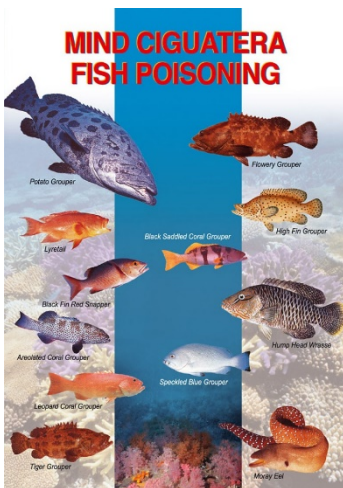
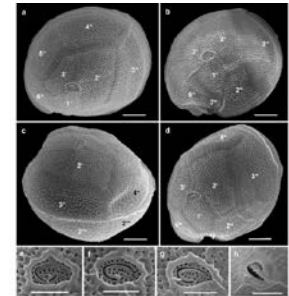


“RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE”



71st Advisory Forum Meeting
03-04 April 2019
Bucharest, Rumania

- **Ciguatoxin** is produced by micro algae, or dinoflagellates, called *Gambierdiscus* spp. The toxins climb up the food chain until the contaminated fish are caught and served to people.

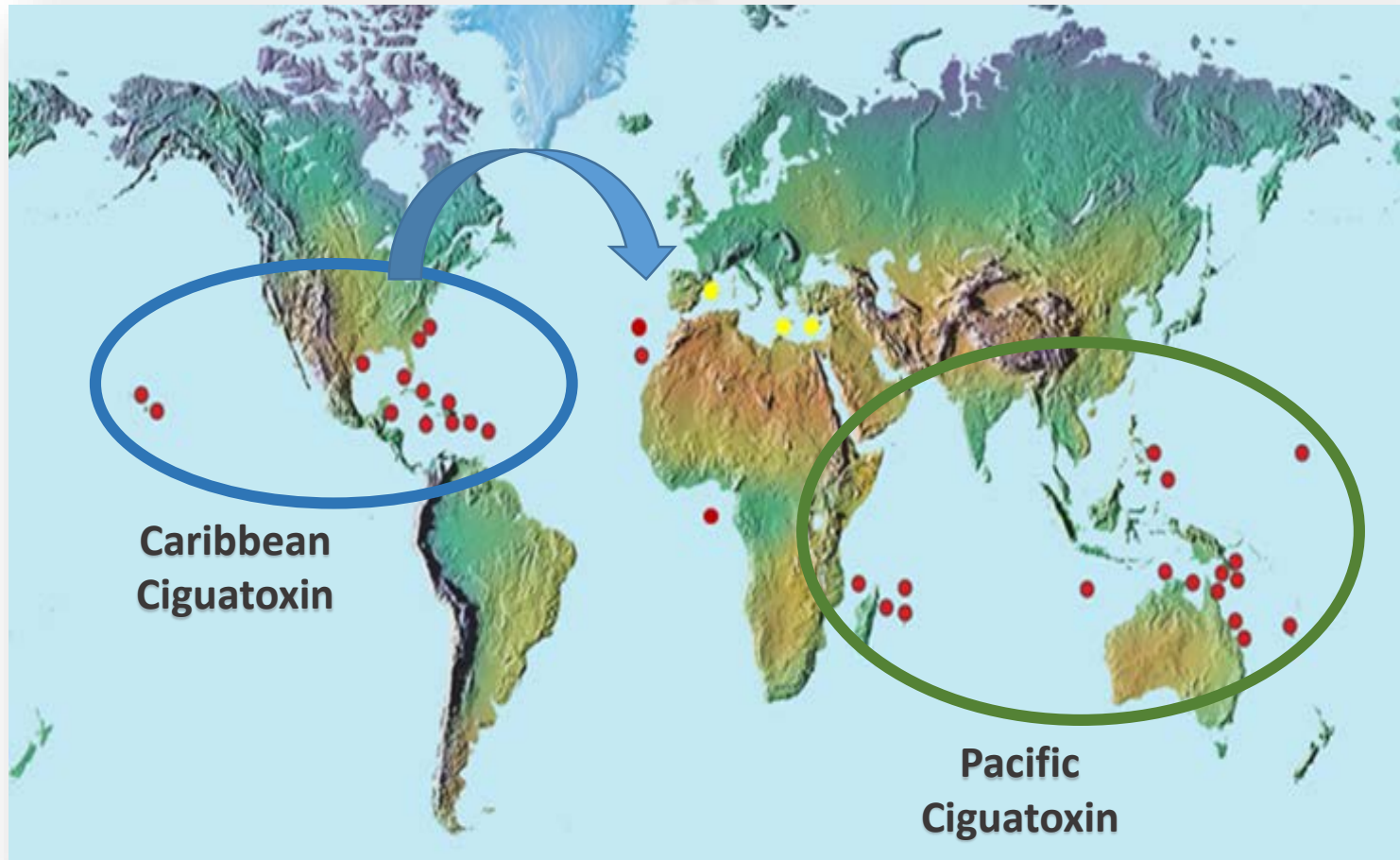


- These toxins cause **Ciguatera food poisoning (CFP)** which is a complex syndrome: **gastrointestinal, neurological and cardiovascular effects**. CFP is primarily associated with the **consumption of large predator fish that have accumulated the toxins** by feeding on smaller contaminated coral reef fish.

- At present, **CFP is the most common type of marine biotoxins food poisoning worldwide** with an estimated number of **10.000 to 50.000 people** suffering from the disease annually.

Ciguatera Fish Poisoning





Red dots: ciguatera cases of poisoning. Yellow dots: presence of Gambierdiscus spp.

2004 → *Gambierdiscus* spp., responsible for **ciguatera** in the waters of the Canary Islands and Madeira microalgae, was detected.

2008 → autochthonous ciguatera outbreaks in **Spain** (Canary Islands) and in **Portugal** (Madeira).



Between **2008 and 2018** → 18 outbreaks (115 cases) recorded in Canary Islands

Fish *genus* ***Seriola*** was involved in many of the outbreaks.

These new findings suggest the microorganism is becoming an increasing risk for European countries.



RASFF Portal

European Commission > RASFF Portal

Notifications list | New search | Export to... ▾

Search result: 9 notifications

Search criteria: **Subject *CIGUATOXIN*** OR ***CIGUATERA***

First | Previous 100 | Next | 9 of 9

Acanthocymium solandri from Vietnam

Classification	Date of case	Reference	Notifying country	Subject					
1. alert	05/03/2019	2019.0875	France	ciguatera poisoning suspected to be caused by frozen wahoo loin fillets(<i>Acanthocymium solandri</i>) from Vietnam	fish and fish products	food	serious	Details	
2. information for attention	27/07/2017	2017.1112	France	ciguatoxins in frozen red snapper fillets (<i>Lutjanus spp</i>) from India	fish and fish products	food	serious	Details	
3. alert	17/03/2017	2017.0345	Germany	ciguatera poisoning suspected to be caused by frozen red snapper fillets (<i>Lutjanus bohar</i>) from Vietnam, via Denmark	fish and fish products	food	serious	Details	
4. information for attention	22/08/2016	2016.1155	France	ciguatoxins in chilled kingfish (<i>Caranx spp</i>) from India	fish and fish products	food	serious	Details	
5. information for attention	22/08/2016	2016.1152	France	ciguatera poisoning suspected to be caused by frozen red snapper (<i>Lutjanus spp.</i>) from India, via the Netherlands	fish and fish products	food	serious	Details	
6. information for attention	19/08/2016	2016.1152	France	ciguatoxins in wild-caught fish (<i>Caranx spp</i> and others) from Sri Lanka	fish and fish products	food	serious	Details	
7. alert	14/07/2016	2016.0932	France	ciguatera poisoning suspected to be caused by frozen red snapper (<i>Lutjanus spp.</i>) from India, via the Netherlands	fish and fish products	food	serious	Details	
8. information for attention	27/01/2015	2015.0088	France	ciguatoxins in wild-caught fish (<i>Caranx spp</i> and others) from Sri Lanka	fish and fish products	food	serious	Details	
9. alert	16/11/2012	2012.1602	Germany	ciguatera poisoning suspected to be caused by fresh red snapper fillets (<i>Lutjanus spp.</i>) from India	fish and fish products	food	serious	Details	

Lutjanus spp. from: Vietnam and India

- EuroCigua co-funded by the European Food Safety Authority (EFSA) **1 million €**
- **Framework Partnership Agreement** (FPA)
- EuroCigua started on June 1st 2016
- Long-term cooperation between EFSA and **14 partners from six Member States** with the **common general objective** of characterizing the risk of ciguatera food poisoning in Europe.

AECOSAN facilitate the cooperation and Scientific advancement of the Project. The team of this SA integrate the different results of each part of the project, in order to ensure the Scientific coherence and data integration among the different SAs.

MANAGEMENT
AND SCIENTIFIC
COORDINATION

EPIDEMIOLOGY

Due to the ciguatera cases and outbreaks have been reported in Europe since 2008, to determine the incidence and epidemiological characteristic of ciguatera cases and outbreaks in Europe is the main objective of **ISC III**.

The **University of Vigo** is responsible for the characterization of the risk associated of the Ciguatera poisoning, by developing an efficient analytical methodology to identify the toxins, as well as developing standards and reference material to be used for this evaluation and characterization.

CHARACTERIZATION
OF CIGUATOXINS

EVALUATION OF
CIGUATOXINS IN
SEAFOOD AND
ENVIRONMENT

The main objectives of **IRTA** are to evaluate the presence of these toxins in fish and the presence of the potential toxin-producing microalgae *Gambierdiscus* in the environment. This team must to identify fish species which represent a risk for human consumption as well as obtaining primary reference materials containing CTXs.

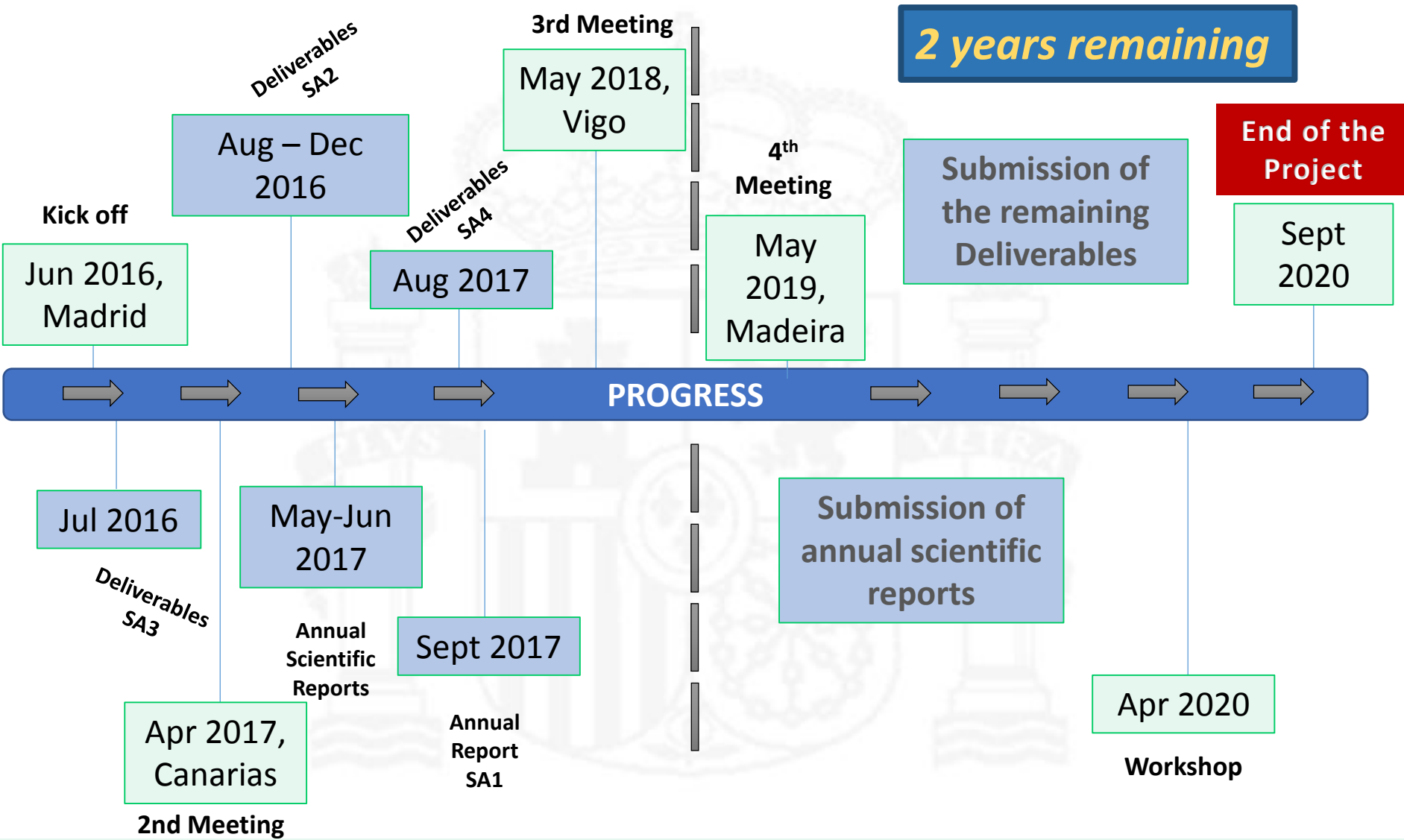
RISK
CHARACTERIZATION
OF CIGUATERA FOOD
POISONING

Coordinator and Partners

	Organisation	Acronym
Coordinator	Agencia Española de Consumo, Seguridad Alimentaria y Nutrición	AECOSAN
Partner 1	Instituto de Salud Carlos III	ISCIII
Partner 2	Institut de Recerca i Tecnologia Agroalimentaries	IRTA
Partner 3	Universidad de Vigo	UVigo
Partner 4	Portuguese Authority for Food and Economic Safety	ASAE
Partner 5	Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P.	–
Partner 6	University of Thessaly	–
Partner 7	Federal Institute for Risk Assessment	BfR
Partner 8	Canary Health Service (Servicio Canario de la Salud)	SCS
Partner 9	Universidad de Las Palmas de Gran Canaria	ULPGC
Partner 10	Instituto Português do Mar e da Atmosfera	IPMA
Partner 11	State General Laboratory (SGL) / Ministry of Health	SGL
Partner 12	French Research Institute for Exploitation of the Sea	IFREMER
Partner 13	Aristotle University of Thessaloniki	–

Collaborators & AB

Collaborators	Ministry of health, Cyprus
	Regional Ministry of Agriculture, Livestock, Fisheries and Water the Canary Islands Government
	Instituto das Florestas e Conservação da Natureza, IP-RAM/ Governo Regional da Madeira, Secretaria Regional do Ambiente e Recursos Naturais
	Direção de Serviços de Investigação e Desenvolvimento da Pesca, Direção Regional de Pescas, Secretaria Regional de Agricultura e Pesca
	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES)
Advisory Board	Dr. Robert Dickey - University of Texas Marine Science Institute
	Dr. Ronald Manger - Fred Hutchinson Cancer Research Center
	Dr. Takeshi Yasumoto - Japan Food Research Laboratories (JFRL)
	European Food Safety Authority - EFSA
	European Centre for Disease Prevention and Control - ECDC
	European Commission (EC)
Joint Research Center (JRC)	



SA2



- ✓ Case definition
- ✓ List of possible data sources for ciguatera cases and outbreaks from each EU MS
- ✓ Surveillance protocol for ciguatera cases and outbreaks
- ✓ Database for collecting cases and outbreaks

SA4



- ✓ Development, optimization and validation of LC-MS/MS for identification and confirmation of CTX

SA3



- ✓ Standardization of the cell-based assay (CBA)
- ✓ Extraction procedures for CTX
- ✓ Description of the protocol literature search and data collection model
- ✓ Sampling strategy *Gambierdiscus* spp. and fish

Case Definition Consensus

future directions, and recommendations for clinicians and patients. It updates and expands upon the previous review of CFP published by Friedman et al. (2008) and addresses new insights and relevant emerging global themes such as climate and environmental change, international market issues, and socioeconomic impacts of CFP. It also provides a proposed universal case definition for CFP designed to account for the variability in symptom presentation across different geographic regions. Information that is important but unchanged since the previous review has been reiterated. This article is intended for a broad audience, including resource and fishery managers, commercial and recreational fishers, public health officials, medical professionals, and other interested parties.

Box 1. Possible universal case definition of ciguatera fish poisoning (CFP).

A case definition is a set of uniform criteria for identifying a disease, which is used for research purposes, clinical diagnosis, or public health surveillance. With regard to CFP, a universal case definition, designed to account for the variability in symptom presentation for fish obtained from different geographic regions (e.g., Caribbean Sea, Indian Ocean and Pacific Ocean), is desirable to help identify cases consistently. Following is a possible case definition. This proposed definition is a refinement or modification of other CFP clinical descriptions or case definitions (e.g., Centers for Disease Control and Prevention's Yellow Book [49], US Food and Drug Administration's Bad Bug Book [51], European Food Safety Authority's Framework Agreement [50]), for global application:

50. European Food Safety Authority (EFSA). Risk characterization of ciguatera food poisoning in Europe: Framework partnership agreement. In Proceedings of the Meeting of the EFSA Advisory Forum, Utrecht, The Netherlands, 8–9 June 2006; Available online: <https://www.efsa.europa.eu/sites/default/files/AF/160608a/160608-p10.pdf> (accessed on 13 March 2017).

Epidemiological data of ciguatera outbreaks reported in Europe since 2012 until 2018

Endemic Fish

Reporting country	Year	No. of Cases	No. Hospitalized	Type of fish	Origin of fish	Place of consumption	CTX detection
Portugal	2012	12	12	<i>Seriola spp.</i> <i>Lachnolaimus maximus</i>	Portugal	Ship at sea	No
Spain	2012	10	0	<i>Seriola spp.</i>	Spain	Restaurant	No Analysis
Spain	2012	9	0	<i>Seriola spp.</i>	Spain	Restaurant	No Analysis
Spain	2012	4	0	<i>Seriola spp.</i>	Spain	Household	No Analysis
Spain	2012	12	0	<i>Epinephelus spp.</i>	Spain	Household	Yes
Spain	2013	16	0	<i>Epinephelus spp.</i>	Spain	Household	Yes
Portugal	2015	7	4	<i>Epinephelus marginatus</i>	Portugal	Restaurant	No
Spain	2015	3	0	<i>Mycteroperca fusca</i>	Spain	Restaurant	No Analysis
Spain	2015	2	0	<i>Pamatomus saltatrix</i>	Spain	Household	Yes
Spain	2015	3	0	<i>Mycteroperca fusca</i>	Spain	Restaurant	No Analysis
Spain	2016	2	0	<i>Epinephelus spp.</i>	Spain	Restaurant	Yes
Spain	2016	3	0	<i>Seriola spp.</i>	Spain	Household	Yes
Spain	2016	2	0	<i>Pagrus pagrus</i>	Portugal	Ship	Yes
Spain	2017	2	0	<i>Epinephelus spp.</i>	Spain	Ship	Yes
Spain	2017	2	0	<i>Mycteroperca fusca</i>	Spain	Ship	Yes
Spain	2018	4	0	<i>Canthidermis sufflamen</i>	Spain	Household	No Analysis

✓ From 2012 to 2018, a total of **93 cases** from **16 ciguatera outbreaks** have been notified from endemic area (Spain and Portugal).

Epidemiological data of ciguatera outbreaks reported in Europe since 2012 until 2018

<i>Imported Fish</i>							
Reporting country	Year	No. of Cases	No. Hospitalized	Type of fish	Origin of fish	Place of consumption	CTX detection
Germany	2012	24	5	<i>Lutjanus bohar</i> <i>L. argentimaculatus</i>	India	Household / Canteen	Yes
Germany	2014	6	1	<i>Lutjanus bohar</i> <i>L. argentimaculatus</i> <i>L. erythropterus</i> <i>Pinjalo pinjalo</i>	Indonesia	Household	Yes
Germany	2015	16	2	<i>Lutjanus bohar</i>	India	Household	Yes
France	2015	2		<i>Lutjanus sp.</i>	Guadeloupe	Household	No
France	2016	7		<i>Lutjanus sp.</i>	Indian Ocean	Household	Yes
Germany	2017	15	2	<i>Lutjanus bohar</i>	Vietnam	Household Restaurant	Yes
France	2017	2		<i>Sparus aurata</i>	-----	Restaurant	No

- ✓ 7 ciguatera outbreaks
- ✓ 72 ciguatera cases

Lutjanus spp.

- ✓ Harmonization of the cell-based assay for CTX detection and set-up of extraction procedures have been development.
- ✓ Sampling of *Gambierdiscus* spp./*Fukuyoa*. spp and the establishment of the desired number of strains has been performed.
- ✓ Environmental parameters were registered (seawater characteristics)
- ✓ Taxonomy for *Gambierdiscus* spp., including the morphological and genetic approach is also undergoing as expected (**Fig. 1 and Fig. 2**).

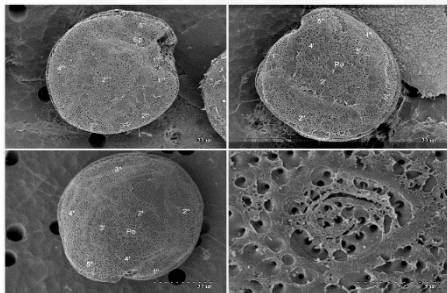


Figure 1. *Gambierdiscus australes* from Menorca, Balearic Island

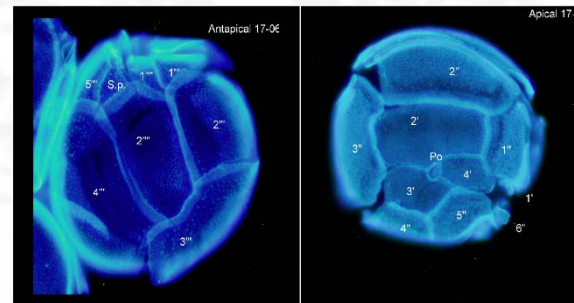


Fig 2. *Gambierdiscus australes*, Lanzarote

- ✓ *Gambierdiscus* was identified in Balearic Island in 2017 and 2018, this confirms the presence of *Gambierdiscus* in the **Western Mediterranean (Fig. 3)**.
- ✓ Cytotoxicity assays showed CTX-like toxicity in *Gambierdiscus* spp strains from the Canary Islands, Crete and the **Balearic Islands**. Further work in strains from other locations has to be achieved.
- ✓ Large-scale cultures (up to 20 L) of some of these strains have been obtained and others are ongoing (SA4).

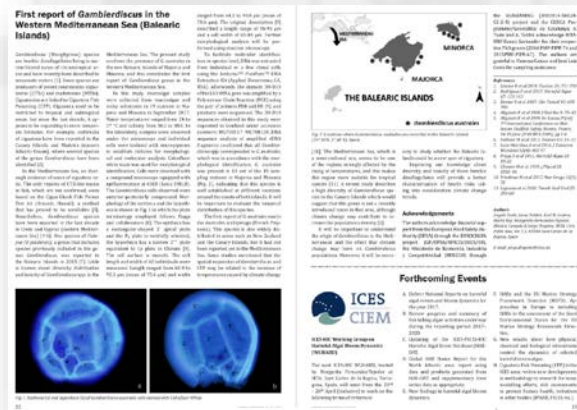
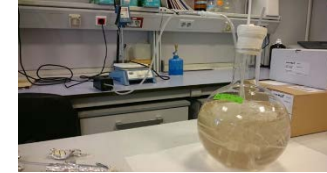


Fig 3. Confirmation of *Gambierdiscus* in Western Mediterranean



	No. Sampling areas	No. Tentative Plan Strains Gambierdiscus	No. Isolated Strains Gambierdiscus	No. Harvested Strains Gambierdiscus	CTX-like toxicity evaluation by N2a cell assay
Canary Islands	7	40	199	68	<ul style="list-style-type: none"> • CTX-like positive: 19 cases (range: 10-499 fg/cell eq.) • CTX-like positive (NQ): 12 cases • CTX-like negative (negative): 1 case
Madeira and Selvagens Islands	3	30	68	1	<ul style="list-style-type: none"> • CTX-like positives: 1 case (83 fg/cell eq.)
Cyprus/ Crete / Samos/ Rhodes	2 / 2	15 / 15	420/ 930 / 37 / 62	2 / 12 / 2 / 20	<ul style="list-style-type: none"> • CTX-like positive: 1 case (4.34 fg/cell eq.) • CTX-like positive (NQ): 12 cases. • CTX-like negative: 4 cases.
Balearic Islands	2	15	197	20	<ul style="list-style-type: none"> • CTX-like positive: 17 cases (range: 1.38 – 104.5 fg/cell eq.) • CTX-like positive (NQ): 3 cases • CTX-like negative (negative): 0 case
TOTAL	16	115	1913	192	

- ✓ Sampling and analysis of fish is also ongoing
- ✓ The cell-based assay has allowed to establish the incidence of fish positive for CTX-like toxicity
- ✓ No positive fish for CTX have been identified in the Mediterranean

	No. Sampling areas	No. Tentative Plan Fish	No. Collected fish	Weight (kg)	<i>CTX-like toxicity (level) by N2a cell assay</i>
Canary Islands	7	525	523 (muscle)		<ul style="list-style-type: none"> • 53 different species of fish (16 showed CTX-like toxicity) • CTX-like positive: 64 cases • CTX-like negative (negative): 452 cases • CTX-like dubious: 7 cases • Pending: 2 samples
Madeira and Selvagens Islands	3	100	70 (2017) + 80 (2018) : 150 fish	0,2 – 300 kg	<ul style="list-style-type: none"> • CTX-like positive: 4 cases (1 amberjack and 3 barred hogfish) • CTX-like negative (negative): 46 cases • CTX-like dubious: 0 cases • Pending: 20+80 = 100 samples
Cyprus	2	70	37 (2017-2018) (74 extracts) Pending 33 fish (2019)	0,3 – 30 kg	<ul style="list-style-type: none"> • CTX-like positive: 0 cases • CTX-like negative (negative): 31 cases • CTX-like dubious: 5 cases • Pending: 38 extracts + 33 samples
Crete	2	70	44 (88 extracts) Pending 26 fish (2019)	0,6 - 12,2 kg	<ul style="list-style-type: none"> • CTX-like positive: 0 cases • CTX-like negative (negative): 58 cases • CTX-like dubious: 0 cases • Pending: 30 extracts + 26 samples
Balearic Islands	2	40	16 (2018) pending 24 fish (2019)	0,7 - 3 kg	<ul style="list-style-type: none"> • CTX-like positive: 0 cases • CTX-like negative (negative): 13 cases • CTX-like dubious: 0 cases • Pending: 27 samples
TOTAL	16	805			

Positive CTX samples from the Canary Islands (May, 2016 - July, 2018).

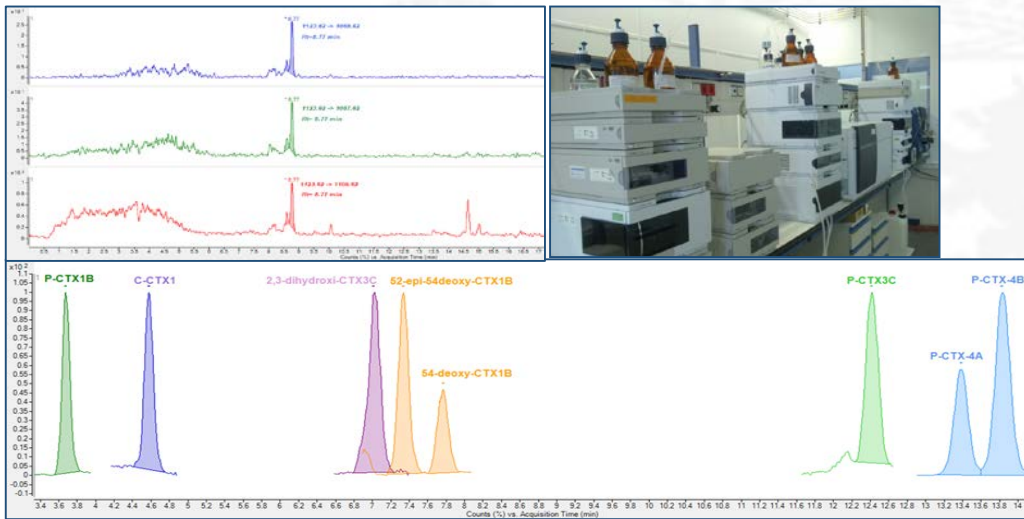
Species	Latin name	No. Samples	Weight (Kg)	CTX-like toxicity (level)
Amberjack	<i>Seriola spp.</i>	10	21.00 - 70.00	Low / Medium-Low / Medium / High
Black moray	<i>Muraena augusti</i>	4	0.40 - 1.03	Medium-Low / Low
Brown moray	<i>Gymnothorax unicolor</i>	1	2.72	High
Common two-banded seabream	<i>Diplodus vulgaris</i>	1	0.32	Medium
Dusky grouper	<i>Epinephelus marginatus spp.</i>	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

✓ Samples related with ciguatera cases & outbreaks

✓ Fish species with higher number of positive samples

The **N2a cell Assay** was standardized and implemented on fish samples. Among **349 fish samples** from the Canary Islands, **9.7% were CTX-like positive**.

After the screening of fish samples from Canary, Madeira and Selvagens Islands, by N2a cell assay in **SG3**, the toxicity of CTX-like positive samples was confirmed by using the optimized LC-MS/MS developed in **SG4**



GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

Detection of ciguatoxins in fish meat: an integrated approach for screening and confirmatory methods

A. Preiß-Weigert¹, A. Gago-Martínez², M. Nicolas³, C. Dell'Aversano⁴

Ciguatoxins (CTXs) and Ciguatera Fish Poisoning (CFP)

- secondary metabolites of the algal genus *Gambierdiscus*
- transformed to CTXs in the marine food chain via herbivorous and carnivorous fish into fish for human consumption

Fig. 1. Determination of parameters to optimize HPLC conditions

- toxicological effects are referred to as ciguatera fish poisoning (CFP)
- symptoms:
 - gastrointestinal
 - neurological (tingling of lips, hand and feet, reversal of temperature sensation)
 - cardiovascular symptoms up to tachycardia

CFP: A Typical of Public Concern

- one of the most common food-borne diseases related to fish consumption
- periodic occurrence in fish from tropical regions e.g. Caribbean and Canary Islands, Pacific and Indian Ocean regions
- CFP cases in fish importing countries due to global trade

International Activities on CTX

- FAO Technical paper 574 (2014) predicted outbreaks of ciguatera poisoning to become one of the increasing food safety threats due to climate change
- Cooper Agreement COOP 2017 analyzed CTXs to be included in the Priority List of Contaminants and Naturally Occurring Toxicants for evaluation by JECFA

Main objectives - Validation of Analytical Methods in order to:

Protect consumers against CFP by:

- strengthening analysis of CTX in fish
- establishing & disseminating the Neuro-2a for CTX screening
- improving availability of CTX standard substances
- CTX confirmation by LC-MS/MS and LC-HRMS
- collection of occurrence data for risk assessment

Promote animal welfare by:

- replacing Mouse Bioassay currently used as a CTX screening tool

Project Module I - CTX-Screening Standardization of N2a Assay

- call for laboratories interested in implementing Neurotoxicity-2a (N2a) Assay
- development of an expert network and organization of meetings to compare different method approaches
- transfer of knowledge and expertise from experienced laboratories e.g. the EURAM to national laboratories establishing N2a Assay

Project Module II - Standard availability Expert Meeting on „State of the Art“

- inventory of available reference standards (Caribbean, Pacific and Indian CTX)
- concept for improvement of standard availability
- support of activities for isolation of CTX from all regions

Project Module III - CTX - Screening by N2a Assay - Collaborative Trial

- collaborative study among the expert network
- preferably with naturally contaminated material depending on availability (otherwise preparation of spiked fish tissue)

Project Module IV - CTX - Confirmation by LC-MS/MS and LC-HRMS

- collection of performance data on methods currently available (expert network)
- scope regarding CTXs from different regions
- performance data (e.g. limits of detection and quantification)
- identification of further requirements to assure reliable quantitative data from LC-MS/MS methods and steps needed to achieve them
- method optimization if necessary
- implementation of the method in laboratories of the network
- collaborative study on toxin identification by LC-HRMS

Important Factors for Project's Success

- laboratories willing to establish Neuro-2a
- partner network who provide/evaluate sample material
- reference material (CTX-standards)
- build-up an activities of current project EuroCigua

Project Proposals

- German Federal Institute for Risk Assessment, Berlin
- European Reference Laboratory for Marine Biotoxins and University of Vigo/Ocean, Department of Analytical and Food Chemistry
- French agency for food, environmental and occupational health safety, Laboratory for Food safety, site of Orleans, Azim
- University of Naples Federico II, Dept. Department of Pharmacy

Fig. 2. Chemical structure of P-CTX1

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

IDENTIFICACIÓN	ESPECIE	Toxicidad IUSA/Vigo*	LC-MS C-CTX1 (ppb) VIGO	Observaciones
EFSA-ULPGC-F0434	Sargo blanco	Inconclusive	n.d.	
EFSA-ULPGC-F0455	Morena picopato	Medium	n.d.	
EFSA-ULPGC-F0457	Peto	Inconclusive	n.d.	
EFSA-ULPGC-F0460	Morena negra	Inconclusive	0.02 ppb	
EFSA-ULPGC-F0462	Medregal	Low	n.d.	
EFSA-ULPGC-F0465	Medregal	Low	n.d.	
EFSA-ULPGC-F0481	Vieja	Inconclusive	n.d.	
EFSA-ULPGC-F0483	Morena picopato	Low	n.d.	Other CTXs?
EFSA-ULPGC-F0487	Medregal	Medium-Low	n.d.	
EFSA-ULPGC-F0488	Mero	Medium-High	0.13 ppb	
EFSA-ULPGC-F0494	Jurel	Medium-Low	n.d.	
EFSA-ULPGC-F0495	Mero	Medium/0.26 ppb*	n.d.	Other CTXs?***
EFSA-ULPGC-F0502	Jurel	Medium/Negative*	n.d.	
EFSA-ULPGC-F0505	Morena pintada	Medium-Low	n.d.	Other CTXs?
EFSA-ULPGC-F0509	Medregal	Low	n.d.	
EFSA-ULPGC-F0513	Mero	Medium/0.23 ppb*	n.d.	Other CTXs?
EFSA-ULPGC-F0516	Morena negra	Low	n.d.	
EFSA-ULPGC-F0517	Morena pintada	Low	<LOQ (0.015 ppb)	
EFSA-ULPGC-F0527	Seiffa	Medium	<LOQ (0.015 ppb)	
EFSA-ULPGC-F0528	Morena negra	Medium-Low	n.d.	Other CTXs?
EFSA-ULPGC-F0534	Sargo breado	Medium-Low	n.d.	Other CTXs?
EFSA-ULPGC-F0555	Morena picopato	Medium	<LOQ (0.015 ppb)	
EFSA-ULPGC-F0556	Peto	Inconclusive	n.d.	
EFSA-ULPGC-F0563	Medregal	Medium-Low	n.d.	
EFSA-ULPGC-F0565	Medregal	Low	n.d.	

*N2a Vigo

***C-CTX + H₂O, or 52,56,56-trihydroxy-N-seco-C-CTX1

Canary Islands

IDENTIFICACIÓN Control Oficial	ESPECIE	Toxicidad IUSA/Vigo*	LC-MS C-CTX1 (ppb) VIGO	Observaciones	SELECCIONADO SG4 (SÍ/NO)
3248	Peto	Baja	n.d.		No
3342	Medregal	Alta/ 0.56 ppb*	n.d.	Other CTXs?	
3354	Medregal	Alta/ 0.32 ppb*	n.d.	Other CTXs?	
3370	Medregal	Alta/ 0.43 ppb*	n.d.	Other CTXs?	
3276	Medregal	Alta/ 0.5 ppb*	n.d.	Other CTXs?***	
3277	Medregal	Alta/ 0.38 ppb*	n.d.	Other CTXs?	
3385	Mero	Alta	0.24 ppb		Si
3394	Medregal	Alta	0.02 ppb	Other CTXs?	
3034	Mero	Alta/ 0.13 ppb*	<LOQ (0.015 ppb)	Other CTXs?	
3436	Mero	Alta	0.2 ppb	Other CTXs?***	Si
3442	Medregal	Alta/ 0.7 ppb*	n.d.	Other CTXs?	

* N2a Vigo

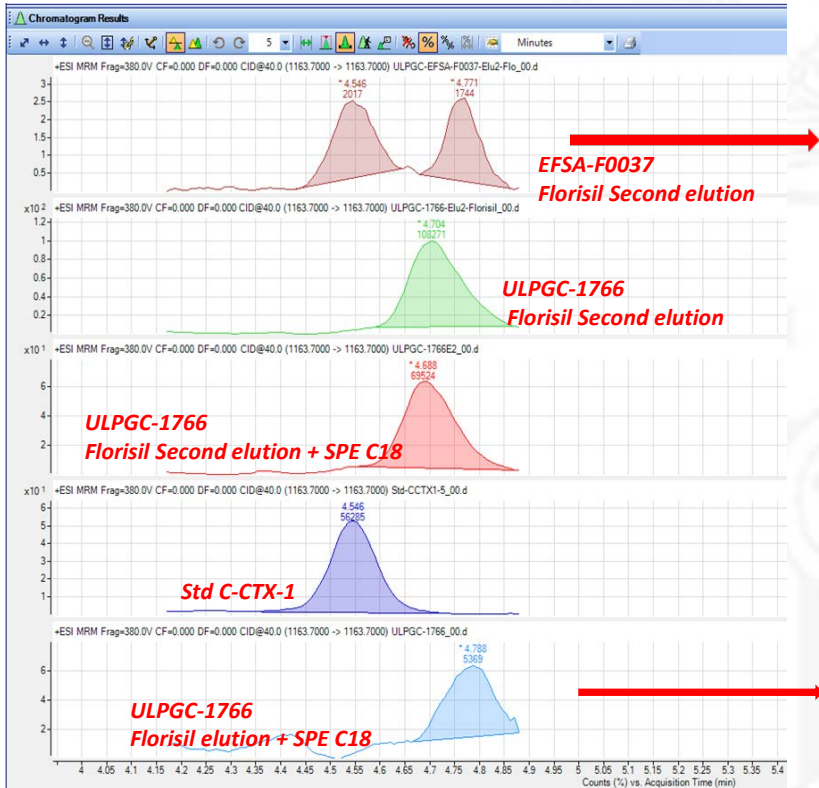
** D) Pottier et al 2002

***C-CTX + H₂O, or 52,56,56-trihydroxy-N-seco-C-CTX1

Sample	Latin Name	N2a Vigo	C-CTX1		Other CTXs - Analogues	Observations
		C-CTX1 eq (ng/g)	Method A	Method B		
			Quantitation (ng/g)	Confirmation		
IPMA-1	Bodianus scrofa	0,29	0,20	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-2	Bodianus scrofa	0,12	0,11	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-3	Bodianus scrofa	0,44	0,23	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-5	Diplodus cervinus	0,55	n.d.	n.d.	C-CTX-1159? & C-CTX-1157 (Pottier et al 2002, Abraham et al 2012)	
IPMA-9	Sphyraena viridensis	0,9	0,14	Yes	n.d.	High Matrix Effect in LC-MS
IPMA-10	Bodianus scrofa	0,1	0,14	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-25	Bodianus scrofa	0,59	0,48	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-42	Bodianus scrofa	1,1	0,49	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-46	Balistes capriscus	0,29	0,09	n.d.	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-55	Bodianus scrofa	0,14	0,1	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-56	Bodianus scrofa	0,21	0,19	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-57	Bodianus scrofa	0,13	0,14	Yes	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	
IPMA-58	Sphyraena viridensis	0,4	<LOQ	n.d.	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	High Matrix Effect in LC-MS
IPMA-70	Bodianus scrofa	0,18	0,08	n.d.	C-CTX-1159? (Pottier et al 2002, Abraham et al 2012)	

- ✓ Hotspot in Selvagens: 28 total samples; **14 positive samples** -> Range: 0,08-0,49 ppb (C-CTX1)
- ✓ 11 CTX contaminated samples > 0,1 ppb

Confirmation of possible CTXs analogues



- High toxicity in Cell assay
- C-CTX-1 detected by LC-MS/MS
- “Interference” (same M/Z, dif. Ret. time) in the second elution of the Florisil
- Ret. time of the “interference” similar to the one present in UPLGC-1766

- High toxicity in Cell assay
- C-CTX-1 **not detected** by LC-MS/MS
- An “Interference” (same m/z, dif Ret time) was detected after complete SPE (Florisil and C18), also detected in the second elution of Florisil

CTXs analogues with similar m/z but different polarity seem to be present in some samples

SA1

- SA1 provided and managed the necessary tools to facilitate good communications among the partners of the project and with the collaborators, experts and other stakeholders.
- Data integration, based on the tasks described in the main objectives.

SA2

- Case definition
- An epidemiological surveillance protocol for ciguatera in the EU. Included two questionnaires.
- From 2012 to 2018, 167 cases from 23 ciguatera outbreaks have been notified in Spain, Portugal, Germany and France.

SG3

- *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.

SG4

- An implemented LC-MS/MS method for the confirmation of the presence of CTX was developed
- Caribbean CTX in particular C-CTX1 and some potential analogues have been characterized as the main responsible for the CTX contamination of fish from Canary Islands and Madeira
- Fish contaminated with CTXs have been selected and are being process to prepare Reference materials

Leaflet

EUROCIQUA

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 CTX-type populations of *Gambierdiscus* spp.
- To assess the possible presence of CTX in fish, in EU waters;
- Determine the incidence and epidemiological characteristics of ciguatera cases in Europe.

efsa
European Food Safety Authority

ASAE
Asociación Española de Seguridad Alimentaria y Nutrición

BFR
Bundesinstitut für Risikobewertung

IRTA
Institut de Recerca i Tecnologia Alimentària

Ministry of Health

ipema

University of Thessaly

Ministry of Health

IRTA

Agencia Autónoma de Madrilera

Secretaría Regional de Agricultura, Pesca y Caza

Conselleria Regional de Pesca

Conselleria de Agricultura, Ganadería, Pesca y Agua

Departament General de Pesca

UNIVERSIDADE DE VIGO

GOBIERNO DE CANARIAS

Conselleria de Agricultura, Ganadería, Pesca y Agua

Departament General de Pesca

ifremer

ARTISTYLE UNIVERSITY OF THESSALY

But Remember:
Eating fish is good for your health!

**CIGUATERA
FOOD POISONING**

GP/EFSA/AFSCO/2015/03

EuroCigua

LEARN MORE ABOUT CIGUATERA
RECOGNIZE THE SYMPTOMS
&
HOW TO PREVENT IT

EuroCigua website

Home | Ciguatera Food Poisoning

Ciguatera Food Poisoning

AN EMERGING RISK IN THE EUROPEAN UNION

Ciguatera food poisoning (CFP) is a type of food poisoning associated with the consumption of seafood with an estimated 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

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

Risk Characterization

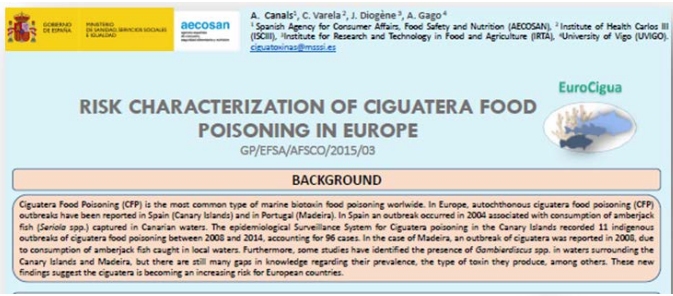
Ciguatera food poisoning (CFP) is the most common type of marine benthic food poisoning worldwide. In Europe, autochthonous CFP outbreaks have been reported in Spain (Balearic Islands) and in Portugal (Madeira). The epidemiological surveillance system for CFP in the Canary Islands received 17 indigenous outbreaks between 2008 and 2010, accounting for 123 cases. In the case of Madeira, an outbreak of the illness was reported in 2010, due to consumption of umbrella fish caught in local waters. Presence of microalgae *Gambierdiscus* spp. has been described in other temperate Mediterranean waters. Other EU countries have experienced outbreaks and cases of ciguatera due to the consumption of contaminated fish imported from non-EU areas. Although there are still many gaps in knowledge regarding the presence of the disease as well as the type of fish involved in the situations, all these findings suggest that CFP is becoming an increasing risk for European consumers.

THE TEAM

The project is funded by the European Food Safety Authority (EFSA). It was launched in June 2015 and will be completed in September 2017. It is implemented through a Framework Partnership Agreement (FPA) comprised of five Independent Specific Agreements (ISA) with specific goals, tasks and deliverables. European focus on the characterization of the risk of Ciguatera Food Poisoning (CFP) in Europe by the establishment of a ciguatera case definition and the identification of data sources for outbreaks and cases of this illness in order to estimate the incidence of the disease in the European Union and the spatiotemporal characteristics of cases. For the characterization of the ciguatera the project will develop outbreak and viable methods for the detection, identification and confirmation of the presence of ciguatera in the context of microalgae and in contaminated fish samples in temperate waters and finally characterize the chemical structures of CTX-type toxins. EuroCigua Project will coordinate a task for collaboration: identify all laboratories, agencies and institutions related to CFP in Europe or worldwide.

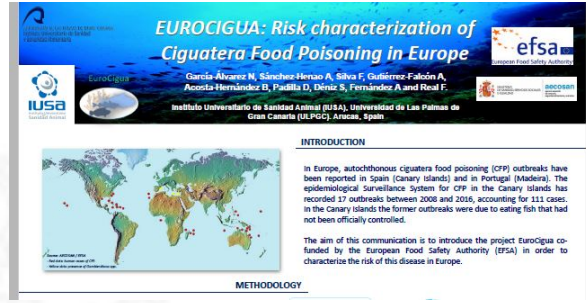
BUILD UP AWARENESS

- English
- Portuguese
- Spanish
- German
- French
- Greek
- Italian



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

BACKGROUND
 Ciguatera Food Poisoning (CFP) is the most common type of marine biotoxin food poisoning worldwide. In Europe, autochthonous ciguatera food poisoning (CFP) outbreaks have been reported in Spain (Canary Islands) and in Portugal (Madeira). In Spain an outbreak occurred in 2004 associated with consumption of amberjack fish (*Girella spp.*) captured in Canarian waters. The epidemiological Surveillance System for Ciguatera poisoning in the Canary Islands recorded 11 indigenous outbreaks of ciguatera food poisoning between 2008 and 2014, accounting for 96 cases. In the case of Madeira, an outbreak of ciguatera was reported in 2006, due to consumption of amberjack fish caught in local waters. Furthermore, some studies have identified the presence of *Gambierdiscus* spp. in waters surrounding the Canary Islands and Madeira, but there are still many gaps in knowledge regarding their prevalence, the type of toxin they produce, among others. These new findings suggest the ciguatera is becoming an increasing risk for European countries.



EUROCIQUA: Risk characterization of Ciguatera Food Poisoning in Europe

García-Alvarez M, Sánchez-Hernao A, Silva F, Gutiérrez-Falcón A, Acosta-Hernández D, Padilla D, Díez S, Fernández A and Real F.

Instituto Universitario de Sanidad Animal (IUSA), Universidad de Las Palmas de Gran Canaria (ULPGC), Antoxa, Spain.

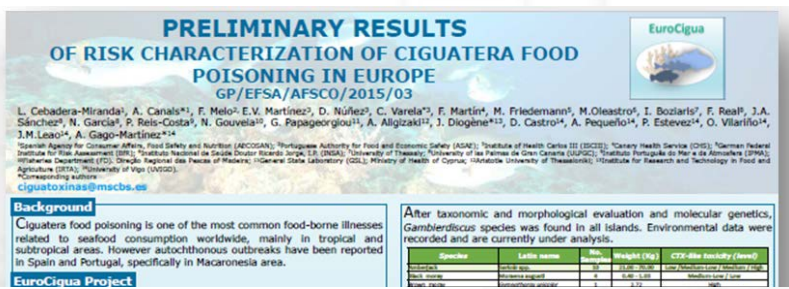
INTRODUCTION
 In Europe, autochthonous ciguatera food poisoning (CFP) outbreaks have been reported in Spain (Canary Islands) and in Portugal (Madeira). The epidemiological Surveillance System for CFP in the Canary Islands has recorded 11 outbreaks between 2008 and 2014, accounting for 111 cases. In the Canary Islands the former outbreaks were due to eating fish that had not been officially controlled.

The aim of this communication is to introduce the project EuroCigua co-funded by the European Food Safety Authority (EFSA) in order to characterize the risk of this disease in Europe.

METHODOLOGY

Communication presented in The ECSafeSEAFOOD final conference in Brussels, Jan - 2017

Communication presented in The 18th International Conference on Diseases of Fish and Shellfish, was held 4th to 8th September, 2017 in Belfast, United Kingdom.



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

L. Cebadera-Miranda¹, A. Canals², F. Melo³, E.V. Martínez⁴, D. Núñez⁵, C. Varela⁶, F. Martín⁷, M. Friedemann⁸, M. Oleastro⁹, I. Bozlaris¹⁰, F. Real¹¹, J.A. Sánchez¹², N. García¹³, P. Reis-Costa¹⁴, N. Gouveia¹⁵, G. Papageorgiou¹⁶, A. Alizizaki¹⁷, J. Diogène^{18,19}, D. Castro²⁰, A. Pequeño²¹, R. Estevez²², O. Villarino²³, I.M. Leao²⁴, A. Gago-Martínez²⁵

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

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	Sex	Weight (kg)	CTX-like toxicity (nmol)
Amberjack	<i>Girella nigricans</i>	♂	0.5	0.1
Amberjack	<i>Girella nigricans</i>	♀	0.5	0.1
Amberjack	<i>Girella nigricans</i>	♂	0.5	0.1

8th International Symposium on Recent Advances in Food Analysis (RAFA) 7-10 November 2017, Prague (Czech Republic)

- Poster: Application of cell based assay for the screening of emerging marine biotoxins in Europe.

Communication presented in the Scientific Conference of EFSA, Parma, Sept – 2018

International Conference on Food Contaminants (ICFC), 13-14 July 2017, Braga (Portugal)


- Poster: Application of N2a assay on the analysis of emerging marine biotoxins and further confirmation by LC-MS/MS.

40th International Conference on Environmental & Food Monitoring (ISEAC-40), 19-22 June 2018, Santiago de Compostela (Spain)


6th International Symposium “Marine and Freshwater Toxins Analysis”, 22-25 October 2017, Baiona (Spain)

- Poster: Development of alternative procedures for the sample pre-treatment of seafood matrices containing marine biotoxins with lipophilic character.
- Poster: Risk characterization of ciguatera food poisoning in Europe: First steps from the screening to the confirmation.

- Poster: Analysis of Ciguatera fish poisoning toxins, an emergent natural contamination of the marine environment in European coastal areas.



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

Pablo Estevez ^a, David Castro ^a, J. Manuel Leao ^{a, b}, Takeshi Yasumoto ^c, Robert Dickey ^d, Ana Gago-Martínez ^{a, b, d}

^a University of Vigo, Department of Analytical and Food Chemistry, Campus Universitario de Vigo, 36310 Vigo, Spain
^b European Union Reference Laboratory for Marine Biotoxins, CITEXXI, 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



Article

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

Pedro Reis Costa ¹, Pablo Estevez ², David Castro ², Lucía Soliño ¹, Neide Gouveia ³, Carolina Santos ⁴, Susana Margarida Rodrigues ¹, José Manuel Leao ² and Ana Gago-Martínez ^{2,*}



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 (Ciguatera) and ciguatera-like (CL) toxins.

Mediterranean Sea. 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 76-93 µm and a cell width of 65-84 µm. Further morphological analysis will be performed using electron microscopy.

To facilitate molecular identification to species level, DNA was extracted from individual or a few clonal cells using the Arcturus™ PicoPure™ DNA Extraction Kit (Applied Biosystems, CA, USA). Afterwards, the domain D8-D10

Wiley Online Library



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



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

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

Miriam Friedemann ¹

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

Río de Janeiro, Brazil – 3-7 April 2017

CCCF: Ciguatera is a natural occurring toxin in fish and CCCF will be the relevant subsidiary body of CAC to recommend measures to reduce contamination to safe levels to ensure public health and facilitate trade.

37. **The EU informed the Committee of a four-year project co-funded by EFSA and coordinated by Spanish food safety agency (AECOSAN) to determine incidence in Europe of ciguatera fish poisoning and epidemiological incidence cases, assess presence of ciguatoxins in food and environment in Europe, and validate the methods for detection, quantification and confirmation that could contribute to future work on ciguatoxins.**

Conclusion

38. The Committee:

- agreed to request scientific advice from FAO/WHO to allow the Committee to develop appropriate risk management options;
- noted that the in-session working group on the priority list of contaminants and naturally occurring toxicants for evaluation by JECFA would consider this matter further (see Agenda Item 14).

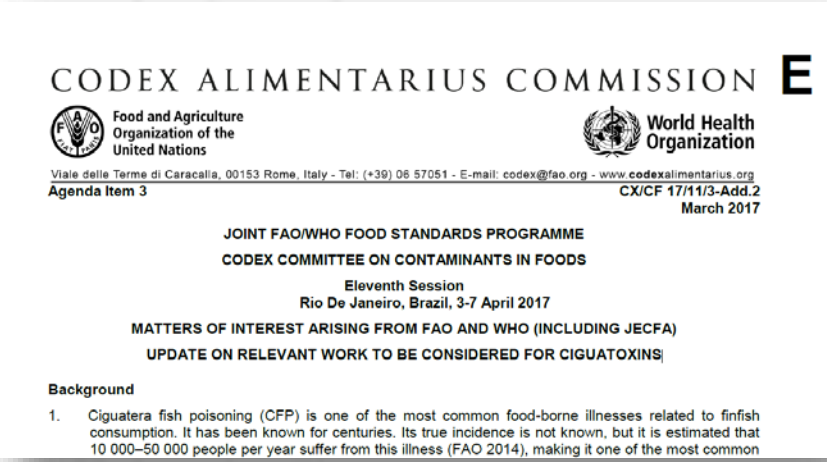
Ciguatera Working Group - FAO

Characterization of the risk of ciguatera worldwide


Experts from **EUROCIGUA** together with other experts including the CODEX experts responsible for the request of risk assessment.

Developing a document that it will include epidemiological and toxicological information, analytical methods, ecology and phytoplankton.

EuroCigua project is mentioned **as a reference project** for the characterization of ciguatera risk in Europe.



CODEX ALIMENTARIUS COMMISSION **E**

 Food and Agriculture Organization of the United Nations

 World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda Item 3 CX/CF 17/11/3-Add.2
March 2017

JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON CONTAMINANTS IN FOODS
Eleventh Session
Rio De Janeiro, Brazil, 3-7 April 2017
MATTERS OF INTEREST ARISING FROM FAO AND WHO (INCLUDING JECFA)
UPDATE ON RELEVANT WORK TO BE CONSIDERED FOR CIGUATOXINS

Background

1. Ciguatera fish poisoning (CFP) is one of the most common food-borne illnesses related to finfish consumption. It has been known for centuries. Its true incidence is not known, but it is estimated that 10 000–50 000 people per year suffer from this illness (FAO 2014), making it one of the most common

Completed Document → Spring of 2019



CIGUATERA FOOD POISONING

The leading non-bacterial food-borne disease associated with consumption of seafood worldwide.

THE PROJECT

This project focuses on the risk characterization of ciguatera food poisoning in Europe.

THE TEAM

The partners belong to fourteen organizations from six Member States.

LATEST NEWS

THIRD SCIENTIFIC CONFERENCE

[Third Scientific Conference "Science, Food, Society"](#)

CALL FOR DATA