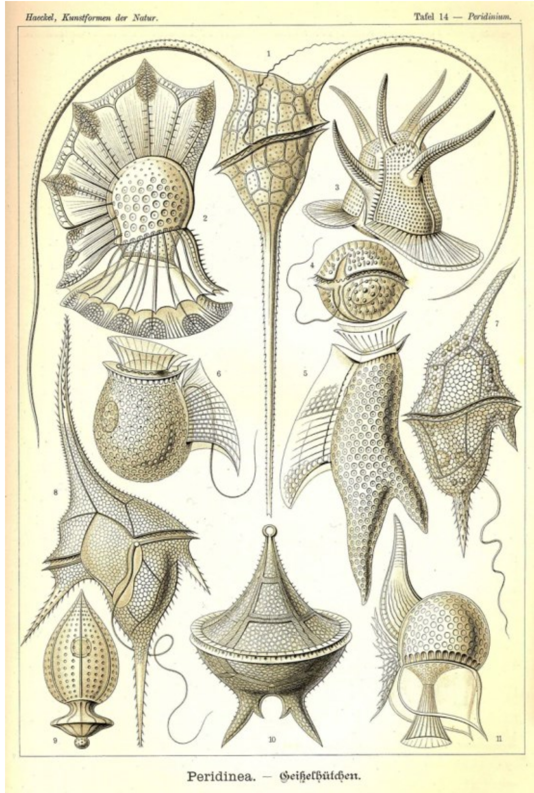
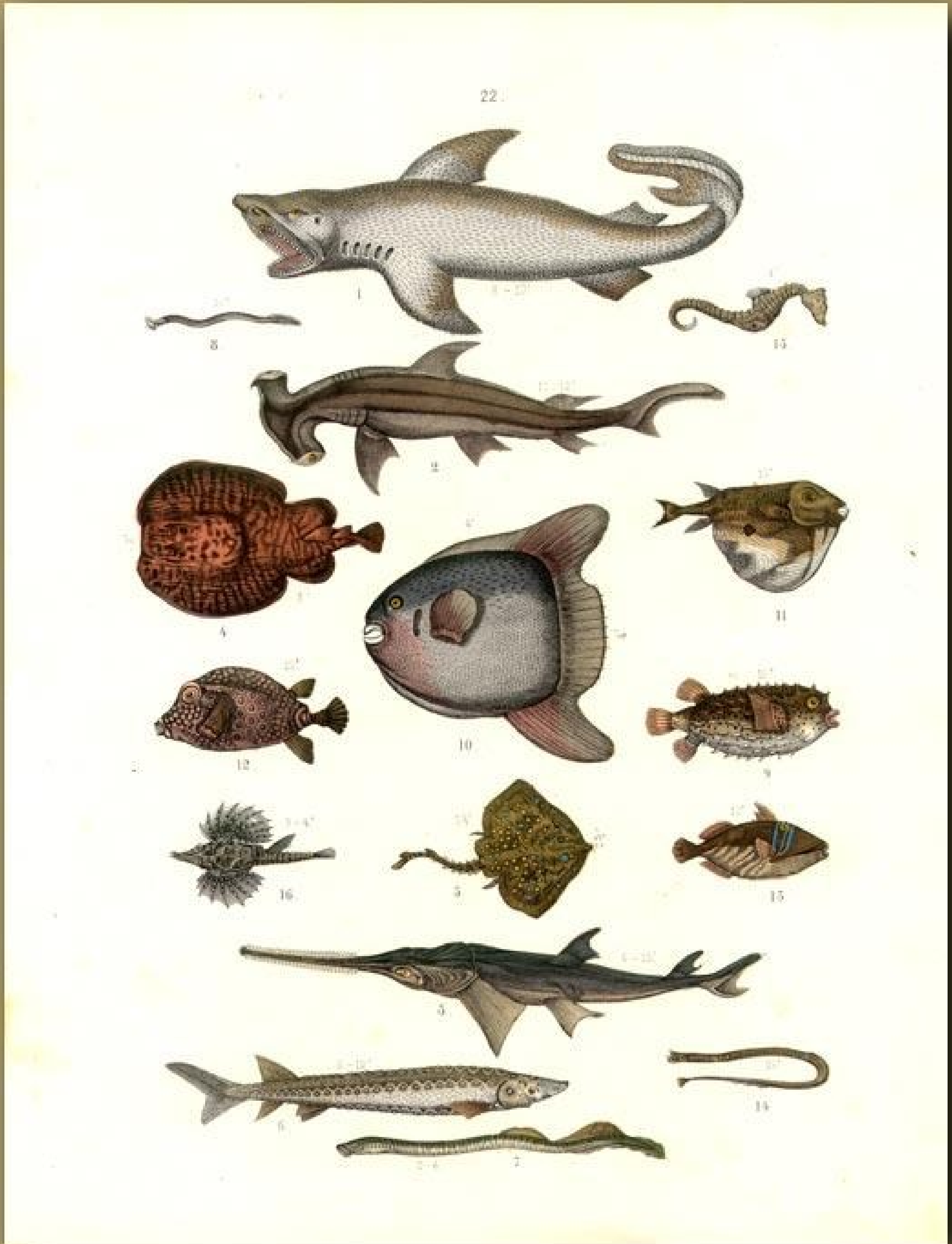
Linking research and surveillance for the risk assessment of emerging marine toxins. Present and future.

Jorge Diogène



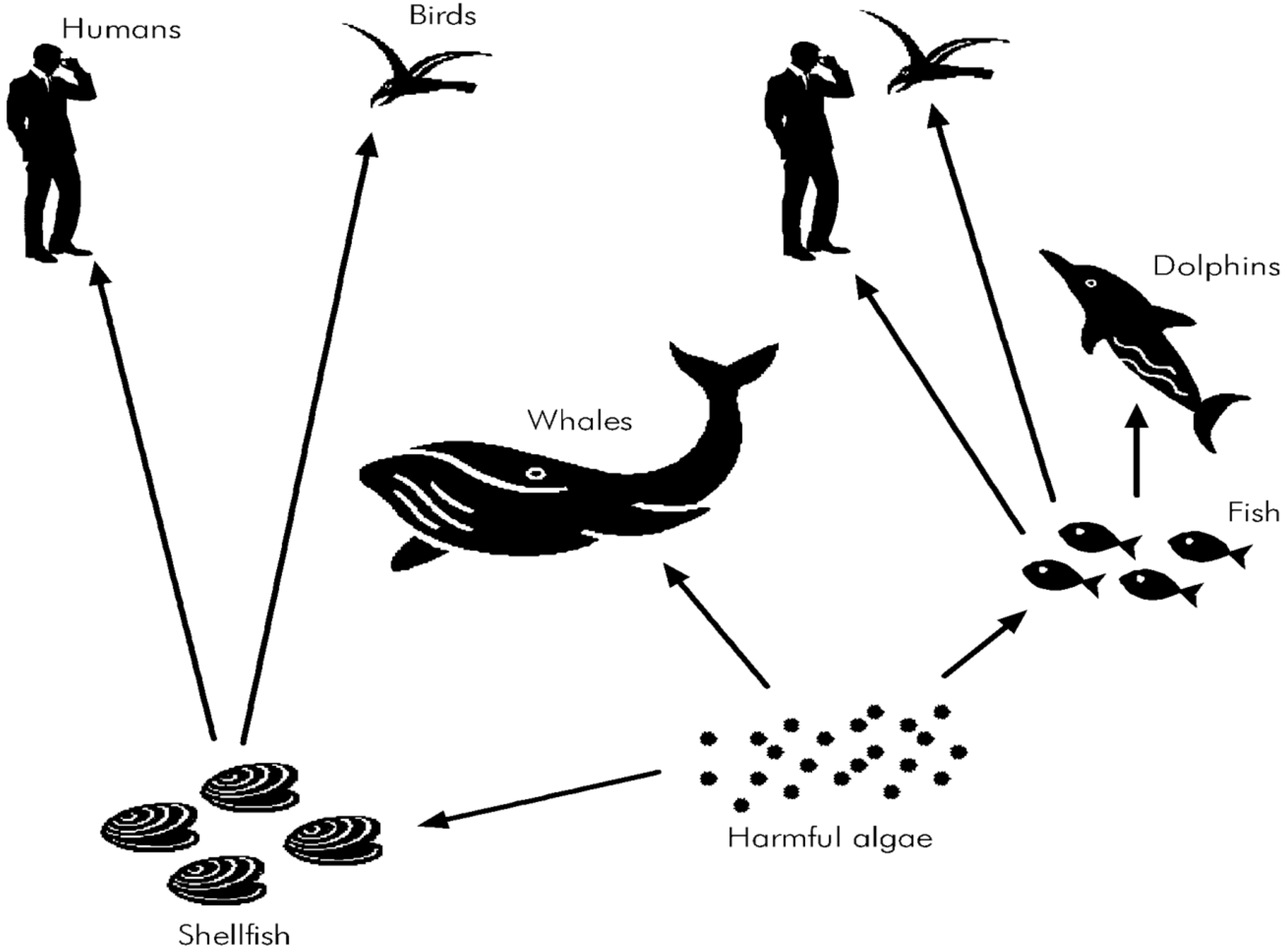


Berlin, 10-11 June 2023

All art has been contemporary. Maurizio Nannucci

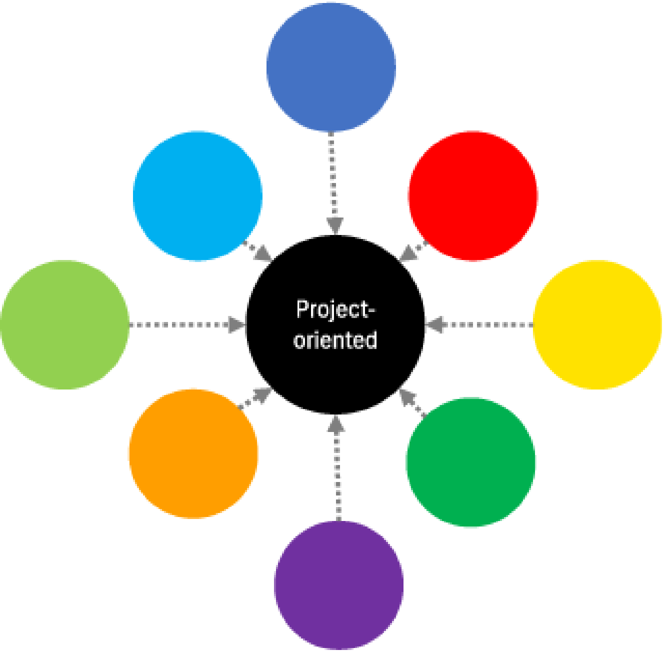
ALL TOXINS HAVE
BEEN AT SOME TIME
“EMERGING TOXINS”

Marine toxins, ASP:



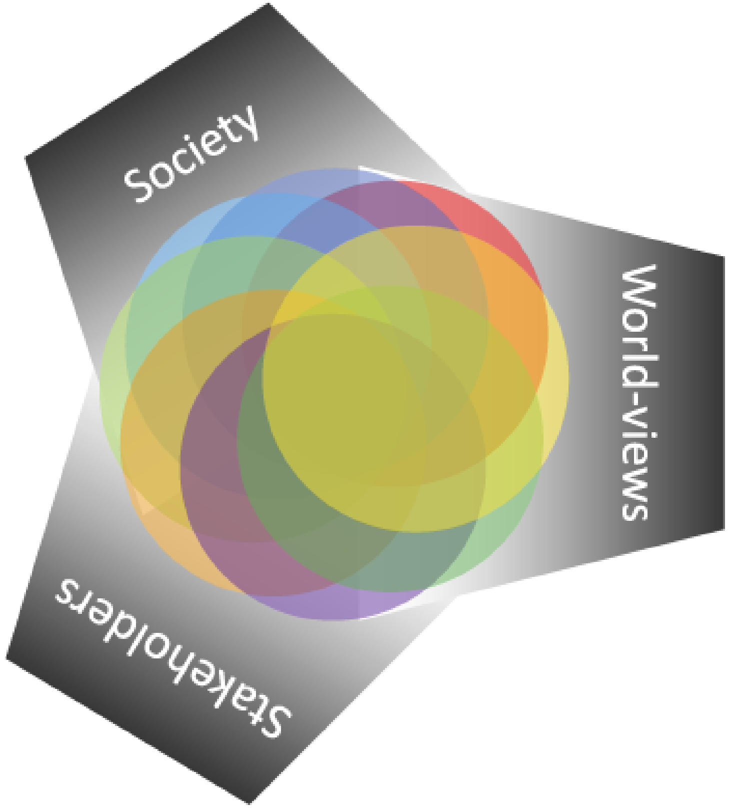
Ciguatera:

Disciplinary, multidisciplinary, interdisciplinary and transdisciplinary strategies



Multidisciplinary

Interdisciplinary



Transdisciplinary

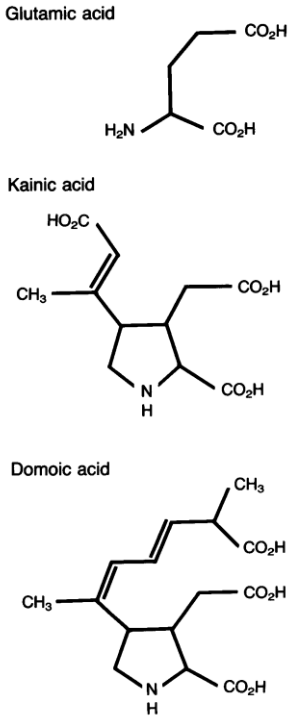
Mayes et al, 2023
Quantitative Plant Biology

ASP, Neurotoxicity of Domoic Acid:

Vol. 322 No. 25 DOMOIC ACID INTOXICATION DUE TO CONTAMINATED MUSSELS — TEITELBAUM ET AL. 1781

NEUROLOGIC SEQUELAE OF DOMOIC ACID INTOXICATION DUE TO THE INGESTION OF CONTAMINATED MUSSELS

JEANNE S. TEITELBAUM, M.D., ROBERT J. ZATORRE, PH.D., STIRLING CARPENTER, M.D., DANIEL GENDRON, M.D., ALAN C. EVANS, PH.D., ALBERT GJEDDE, M.D., PH.D., AND NEIL R. CASHMAN, M.D.



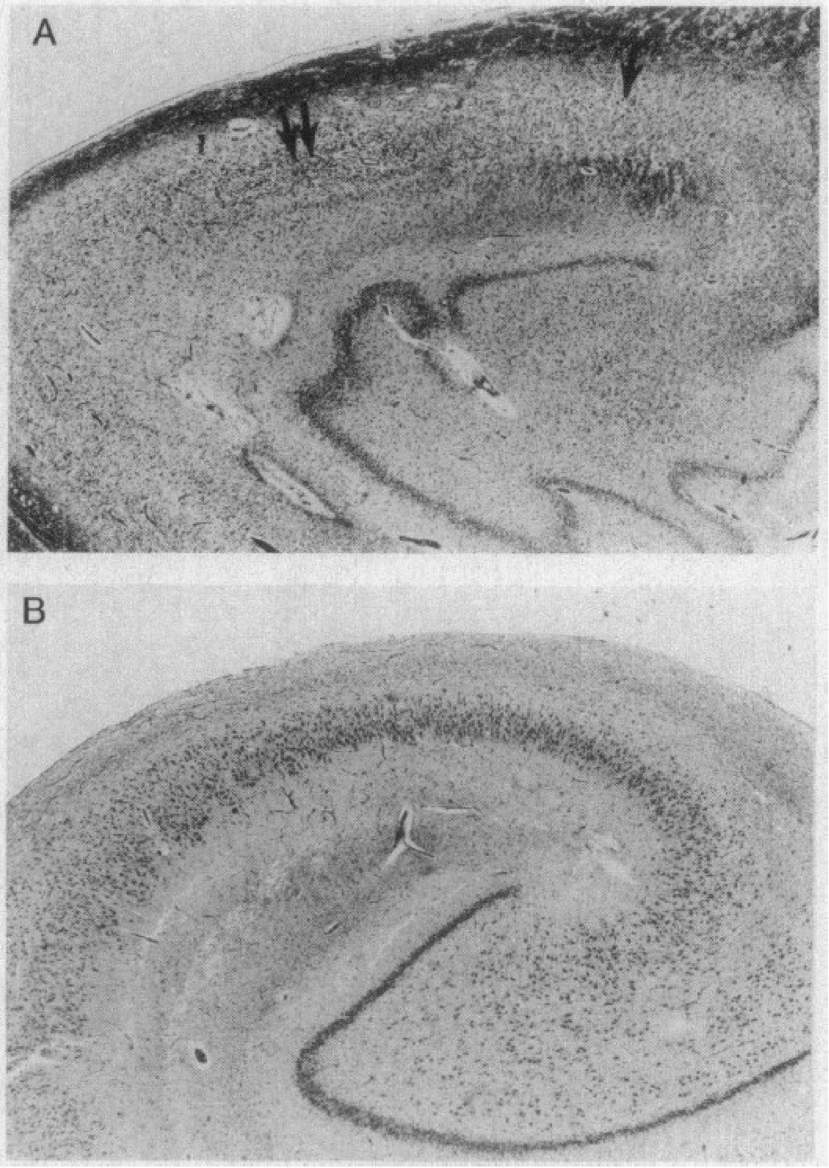


Figure 3. Section of Hippocampus from a Patient Who Died 24 Days after Mussel-Induced Intoxication (Panel A) and from a Normal Subject (Panel B).

In the sample from the patient, there is severe loss of neurons in all fields except H_2 (arrow), and tissue collapse is evident in part of field H_1 (double arrow). Both sections were stained with Luxol fast blue–cresyl violet (×10).

ASP first description:

Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island[1]

J. L. C. Wright,[2] R. K. Boyd, A. S. W. de Freitas, M. Falk, R. A. Foxall, W. D. Jamieson, M. V. Laycock, A. W. McCulloch, A. G. McInnes, P. Odense, V. P. Pathak, M. A. Quilliam, M. A. Ragan, P. G. Sim, P. Thibault, and J. A. Walter

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and

D. Dewar

Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ont., Canada K1A 0S2

Received August 15, 1988

J. L. C. Wright, R. K. Boyd, A. S. W. de Freitas, M. Falk, R. A. Foxall, W. D. Jamieson, M. V. Laycock, A. W. McCulloch, A. G. McInnes, P. Odense, V. P. Pathak, M. A. Quilliam, M. A. Ragan, P. G. Sim, P. Thibault, J. A. Walter, M. Gilgan, D. J. A. Richard, and D. Dewar. Can. J. Chem. **67**, 481 (1989).

The causative agent of toxicity in cultured mussels from a localized area of eastern Prince Edward Island has been identified as domoic acid, a neuroexcitatory amino acid. The toxin was isolated by a number of different bioassay-directed separation techniques including high-performance liquid chromatography, high-voltage paper electrophoresis, and ion-exchange chromatography, and characterized by a number of spectroscopic techniques including ultraviolet, infrared, mass spectrometry, and nuclear magnetic resonance. The isolation and purification methods are described in detail and some new analytical data for domoic acid are reported.

Key words: shellfish toxin, domoic acid, neurotoxin, bioassay-directed analysis.

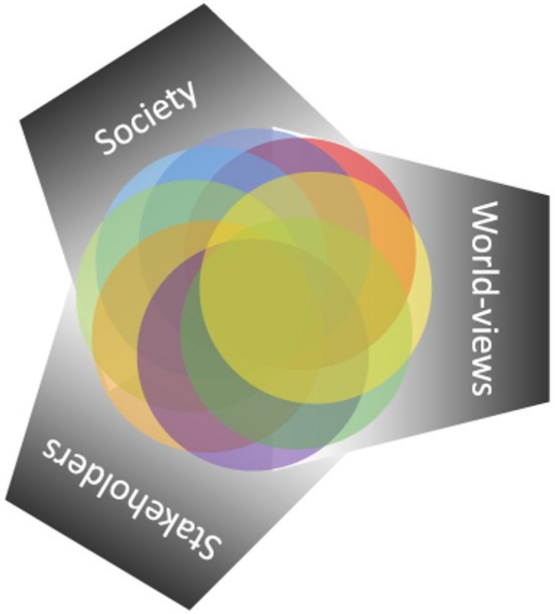
Wright, J. L. C., Boyd, R. K., de Freitas, A. S. W., Falk, M., Foxall, R. A., Jamieson, W. D., Laycock, M. V. et al. 1989. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. Canadian Journal of Chemistry 67(3):481–490.

ASP first description:

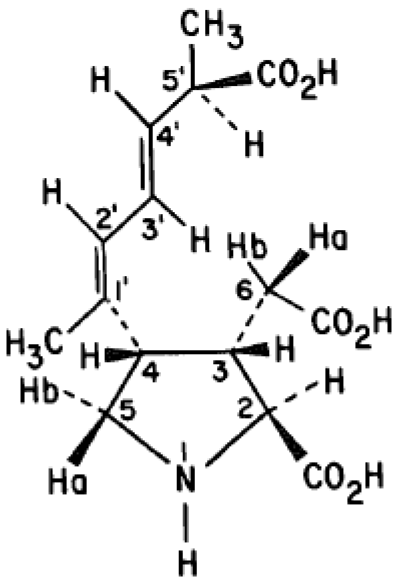
Towards the end of 1987, an outbreak of poisoning in Canada was traced to cultured blue mussels (*Mytilus edulis* L.) from a localized area of eastern Prince Edward Island (P.E.I.). During this period (November 11 to December 12) 153 cases of acute intoxication related to ingestion of toxic mussels were documented (5), corresponding to about three cases per thousand portions of mussels consumed.[3] Symptoms included vomiting and diarrhea, which in some cases were followed by confusion, memory loss, disorientation, and even coma. Three elderly patients died. In the other most severely affected cases neurological symptoms still persist (5). The term Amnesic Shellfish Poison (ASP) has been proposed for this new shellfish toxin.[4]

ASP first description, multi-task force. Transdisciplinarity:

A task force was organized jointly by the Canadian Department of Fisheries and Oceans (DFO) and Health and Welfare Canada (HWC) to establish the extent of the contamination, the chemical nature of the toxin, and its origins. The Atlantic Research Laboratory (ARL) of the National Research Council of Canada, as part of the task force, was one of the laboratories that undertook the task of isolating and determining the chemical nature of the toxin. Within 5 days it was established at ARL that the mollusc toxin was domoic acid, a glutamate agonist (6) originally isolated some 30 years ago from the red alga *Chondria armata* Okamura (7, 8). This paper describes the methods used to isolate and identify domoic acid as the toxin in the contaminated mussels, and reports some new analytical and chemical data on this compound. The possible origin(s) of domoic acid in the 1987 outbreak are also discussed.



ASP first description, Domoic Acid identification:



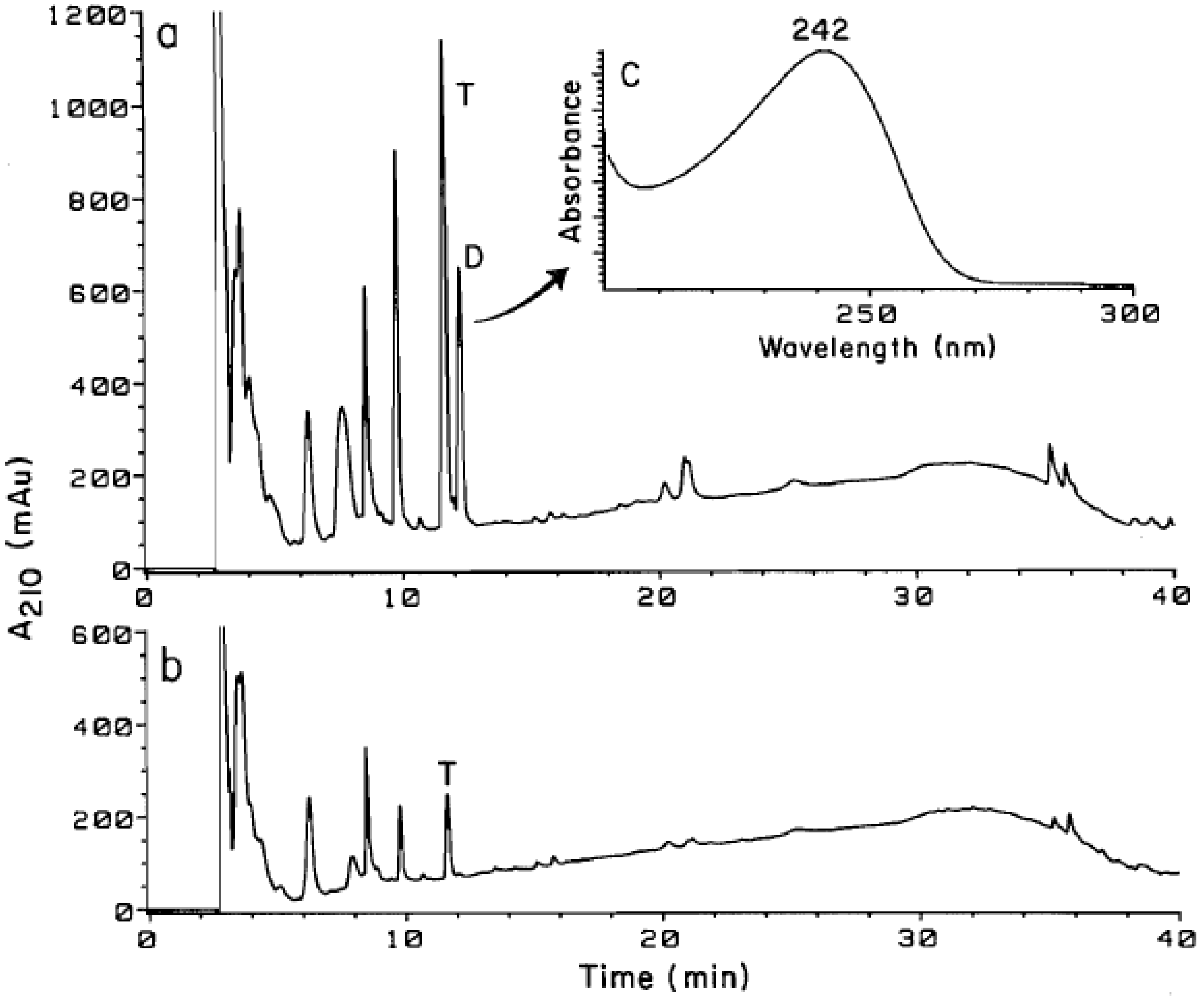


FIG. 1. Reversed-phase hplc chromatograms of XAD-2 cleaned extracts of (a) toxic and (b) control mussels. The inset (c) is the uv–visible spectrum acquired at the apex of peak D (domoic acid). Peak T is due to the amino acid tryptophan. Conditions: 25 cm × 2.1 mm i.d. 5-µm Vydac 201TP52 column at 40°C; 0.2 mL min^{-1} flow rate; gradient elution from TFA/H_2O (0.1:99.9) to CH_3CN/TFA/H_2O (50:0.1:49.9) in 25 min and then to CH_3CN/TFA (99.9:0.1) at 35 min and hold to 50 min; detection provided by absorption at 210 nm (20-nm bandwidth).

ASP first description, Domoic Acid identification:

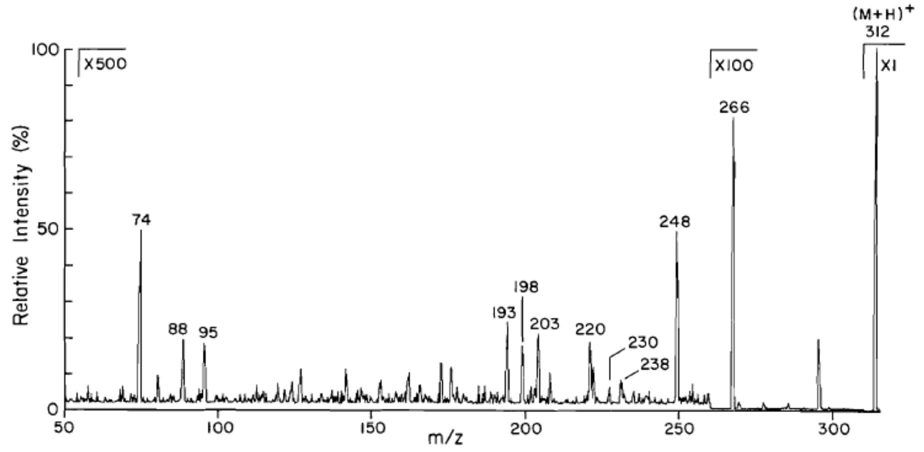


FIG. 3. Fragment-ion mass spectrum, for collision-induced dissociation in rf-only quadrupole, of MH^+ ions (m/z 312) formed by FAB ionization of the toxin in a glycerol/water matrix.

TABLE 1. Nuclear magnetic resonance data for domoic acid

Position	2	3	4	5α	5β	6a	6b	7	$2\text{-}CO_2H$
$\delta(^1H)$, ppm[a,b]	3.98	3.05	3.84	3.49	3.71	2.76	2.50		
Multiplicity[c]	d	dddd	ddd	dd	dd	dd	dd		
J_{HH}, Hz[d]	(2, 3) 8.1	(2, 3) 8.1 (3, 4) 8.4 (3, 6a) 5.8 (3, 6b) 9.1	(3, 4) 8.4 (4, 5α) 7.3 (4, 5β) 7.9	(4, 5α) 7.3 (5α, 5β), −12.2	(4, 5β), 7.9 (5α, 5β), −12.2	(3, 6a), 5.8 (6a, 6b), −16.7	(3, 6b), 9.1 (6a, 6b), −16.7		
% nOe produced when H irradiated[e]	None	H-4, 4.7	H-3, 6.4[g] H-3′, 14.9	H-5β, 12.5 1′-CH_3, 1.0	H-5α, 11.2[g]	H-6b, 5.8	H-6a, 9.0		
$\delta(^{13}C)$, ppm[a,f]	67.1	44.6	42.7	49.1		35.4		177.5	174.9
Multiplicity	bd	bd	bd	bt		bt		dt	dd
J_{CH}, Hz	149 ± 3	138 ± 4	133 ± 4	147.6 ± 2		12 ± 3		3.0 7.2	4.1

Position	1′	2′	3′	4′	5′	6′	1′-CH_3	5′-CO_2H
$\delta(^1H)$, ppm[a,b]		6.13	6.35	5.78	3.30	1.27	1.81	
Multiplicity[c]		d	dd	dd	dq	d	s	
J_{HH}, Hz[d]		(2′, 3′) 11.1	(2′, 3′) 11.1 (3′, 4′) 14.9	(3′, 4′) 14.9 (4′, 5′) 7.8	(4′, 5′) 7.8 (5′, 6′) 7.1	(5′, 6′) 7.1		
% nOe produced when H irradiated[e]		H-4′, 13.0 1′-CH_3, 1.5	H-4, 8.5 H-5′, 4.5	H-2′, 8.0 H-5′, 4.7	H-3′, 3.5 H-4′, 3.0	H-4′, 5.2 H-5′, 10.7	H-5α, 4.5 H-6b, 2.4 H-2′, 13.3	
$\delta(^{13}C)$, ppm[a,f]	133.8	132.8	128.6	135.2	44.9	18.6	23.5	181.9
Multiplicity	bm	bd	dd	bd	bd	qdd	qdd	m
J_{CH}, Hz		151 ± 8	153.5 5.1	161 ± 5	129 ± 4	129.3 ± 1.2 3.9 ± 1.2 3.9 ± 1.2	126.4 ± 1 6.8 5.0	

[a]Reference to TSP (sodium 3-trimethylsilylpropionate-2,2,3,3-D_4) solution in D_2O contained in a concentric tube. Domoic acid solution at pH 3.40 ± 0.05, temperature, 20.0°C.
[b]Error ca. ±0.02 ppm.
[c]s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad.
[d]Error ca. ±0.2 Hz. Spin–spin coupling connectivity confirmed by an ^1H COSY spectrum. Some couplings determined by simulation.
[e]Error ca. ±0.5% for single protons, ca. ±0.15% for methyl groups. Samples were not degassed or otherwise specially prepared for nOe measurements.
[f]^{13}C assignments determined from $^{13}C/^1H$ heterocorrelation experiment and ^1H-coupled ^{13}C spectrum.
[g]The nOe (if any) between H-4 and H-5β was not measurable in these experiments due to the closeness of the resonances.

ASP first description, Domoic Acid identification and toxicity in mouse:

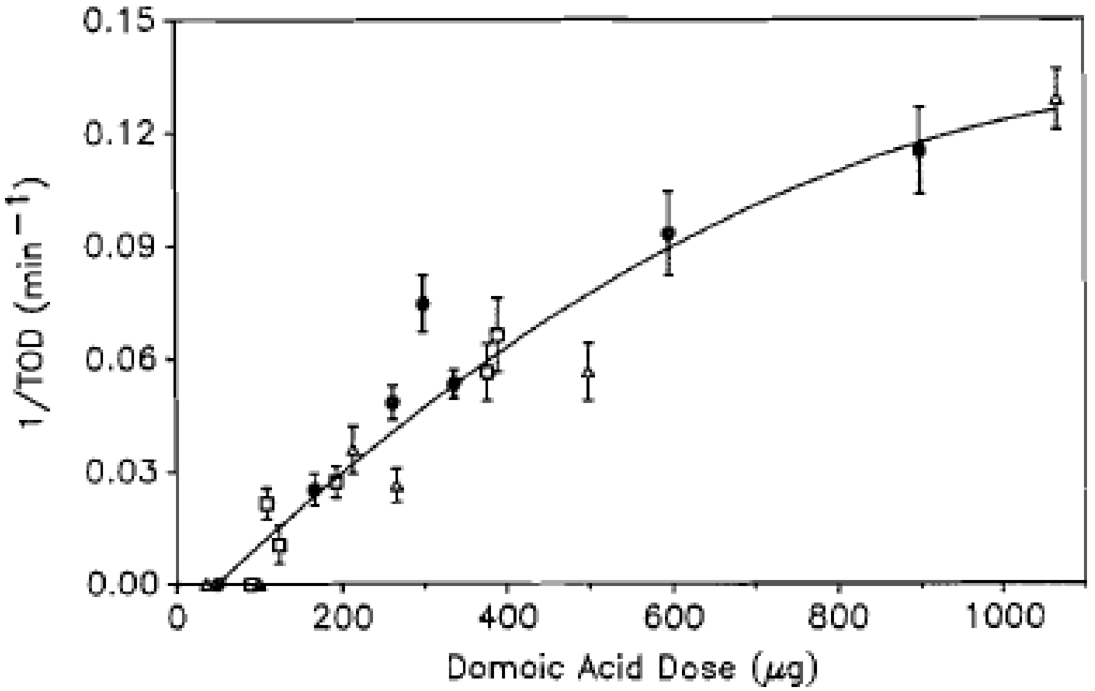
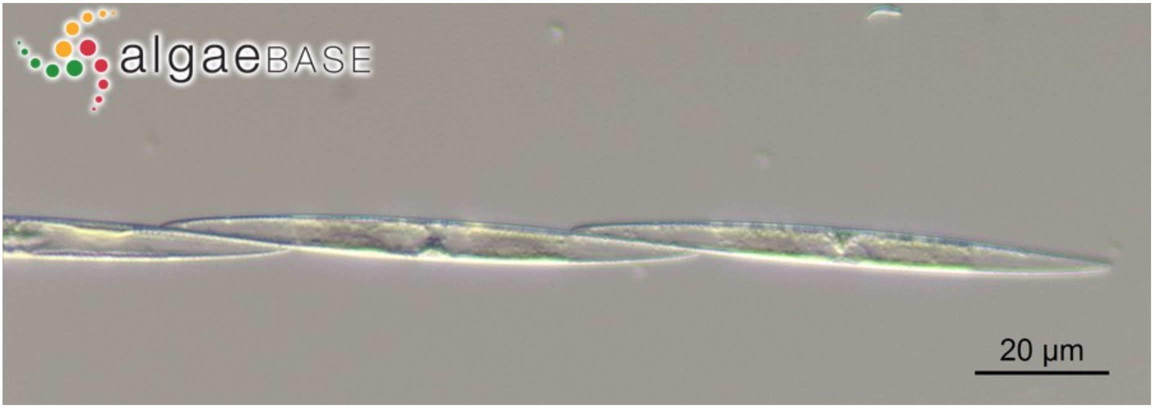


FIG. 4. Toxicity (expressed as reciprocal of time of death (TOD) of mouse) as a function of the amount of domoic acid injected into the mouse (as determined by hplc analysis). The toxicities of room temperature aqueous methanol (△) and boiling 0.1 M HCl (□) extracts of mussel tissue are similar to that of aqueous solutions of pure domoic acid (●). Each point is the mean of 2–4 replicate measurements of TOD for one solution, and the error bars represent an estimate of the standard deviation. The curve is a second-order polynomial least-squares regression on all data points.

ASP first description, phytoplankton link:



species as sources of the toxin in eastern P.E.I. Based on other shellfish toxin results, a more likely source of mussel contamination is the microalgal component of the plankton ingested by filter feeders.

Plankton tows made in early December 1987 by L. A. Hanic (University of Prince Edward Island) revealed an intense diatom bloom in the affected area. This diatom was subsequently identified as *Nitzschia pungens* Grun. f. *multiseries* Hasle.[13] When injected in mice, extracts of the plankton-tow samples produced symptoms identical to those produced by extracts from toxic mussels. As well, all toxic mussels harvested in December 1987 showed engorged digestive glands containing identifiable shell remnants of *N. pungens*; this was also true of toxic mussels harvested in late October and November, although no information is available on the plankton composition at that time. We observed domoic acid in plankton from the affected areas at levels sometimes in excess of 1% dry weight (30), sufficient to account for the levels observed in toxic mussels.

ASP first description, Epidemiology:

AN OUTBREAK OF TOXIC ENCEPHALOPATHY CAUSED BY EATING MUSSELS CONTAMINATED WITH DOMOIC ACID

Trish M. Perl, M.D., Lucie Bédard, M.S.N., Tom Kosatsky, M.D., M.P.H., James C. Hockin, M.D., Ewen C.D. Todd, Ph.D., and Robert S. Remis, M.D., M.P.H.

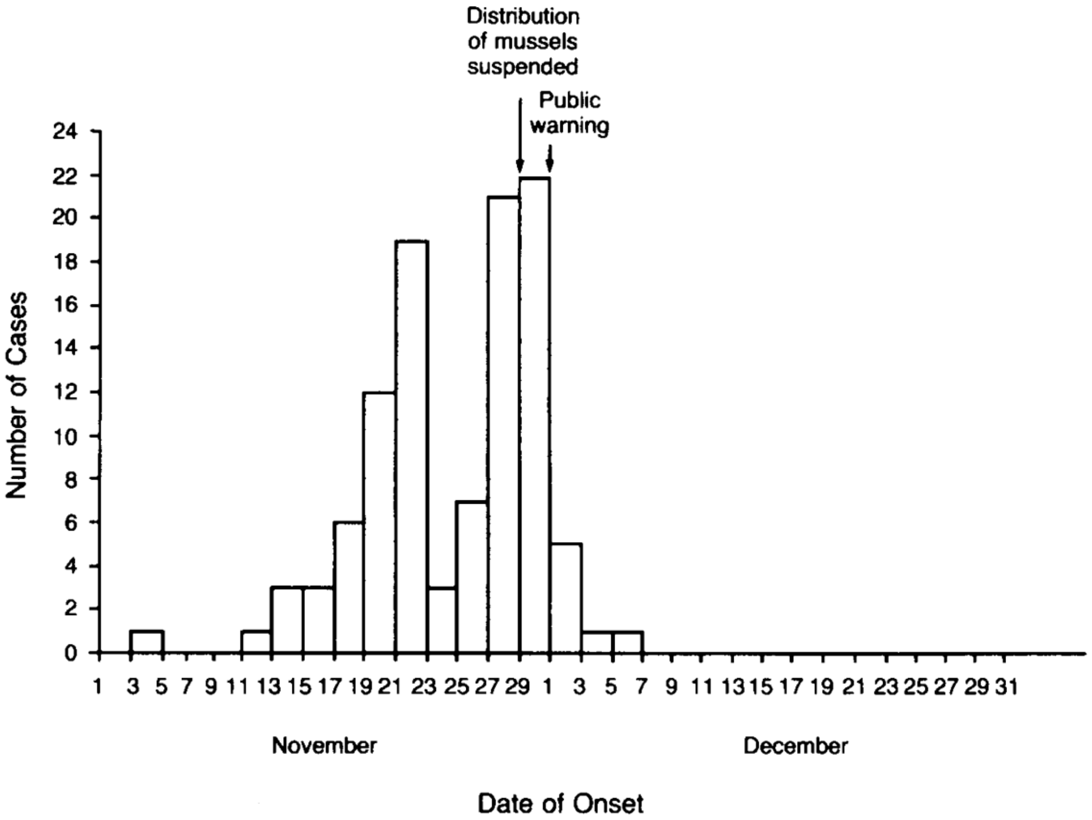


Figure 1. Cases of Mussel-Associated Intoxication, According to the Date of Onset of Symptoms, in Canada in November and December 1987.

A total of 105 cases are shown, since the date of mussel consumption was not known for two patients.

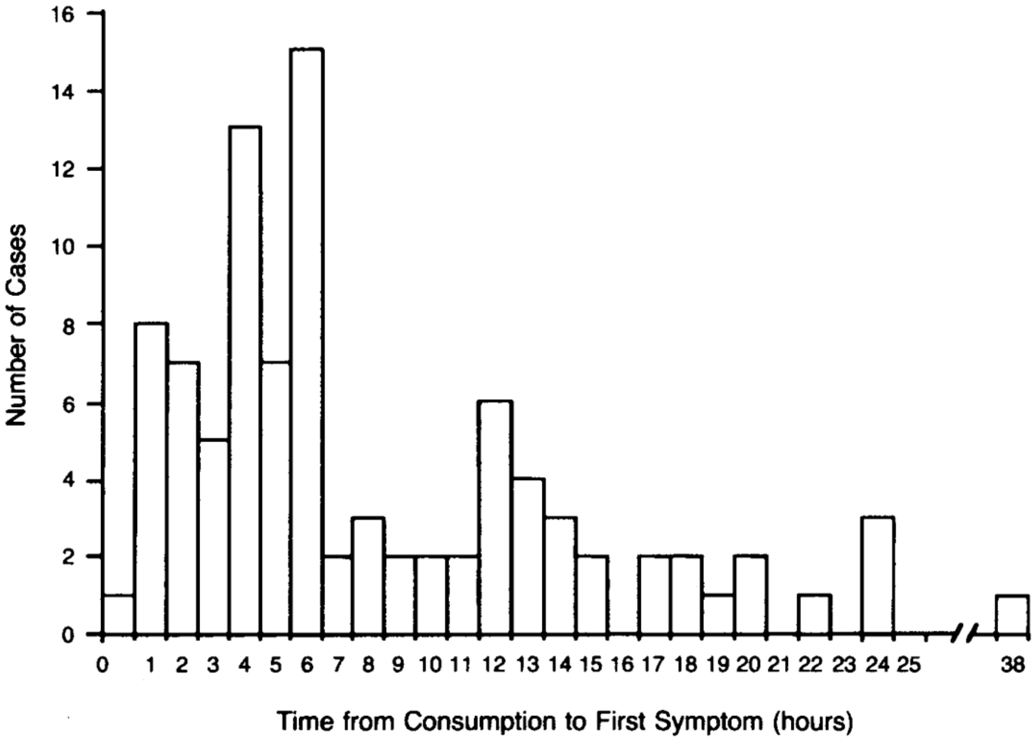


Figure 2. Interval between Mussel Ingestion and the Onset of the First Symptom (Incubation Period).

The data shown are based on responses to 91 questionnaires.

ASP first description, Epidemiology:

Table 1. Symptoms of Illness among 99 Patients after the Consumption of Mussels.*

Symptom	No. of Yes Responses	Total Responses	%
Nausea	75	98	77
Vomiting†	74	97	76
Abdominal cramps†	48	95	51
Diarrhea†	41	97	42
Headache	40	93	43
Memory loss†	24	96	25

*The results were obtained from the standardized questionnaires completed for 99 of the 107 patients. Total responses do not add to 99 because not all questions were answered for each patient.

†Criterion for inclusion as a case.

AN OUTBREAK OF TOXIC ENCEPHALOPATHY CAUSED BY EATING MUSSELS CONTAMINATED WITH DOMOIC ACID

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Table 3. Clinical Course and Estimated Quantity of Domoic Acid Ingested by Nine Patients Who Became Ill after Mussel Consumption and a Control Who Did Not.*

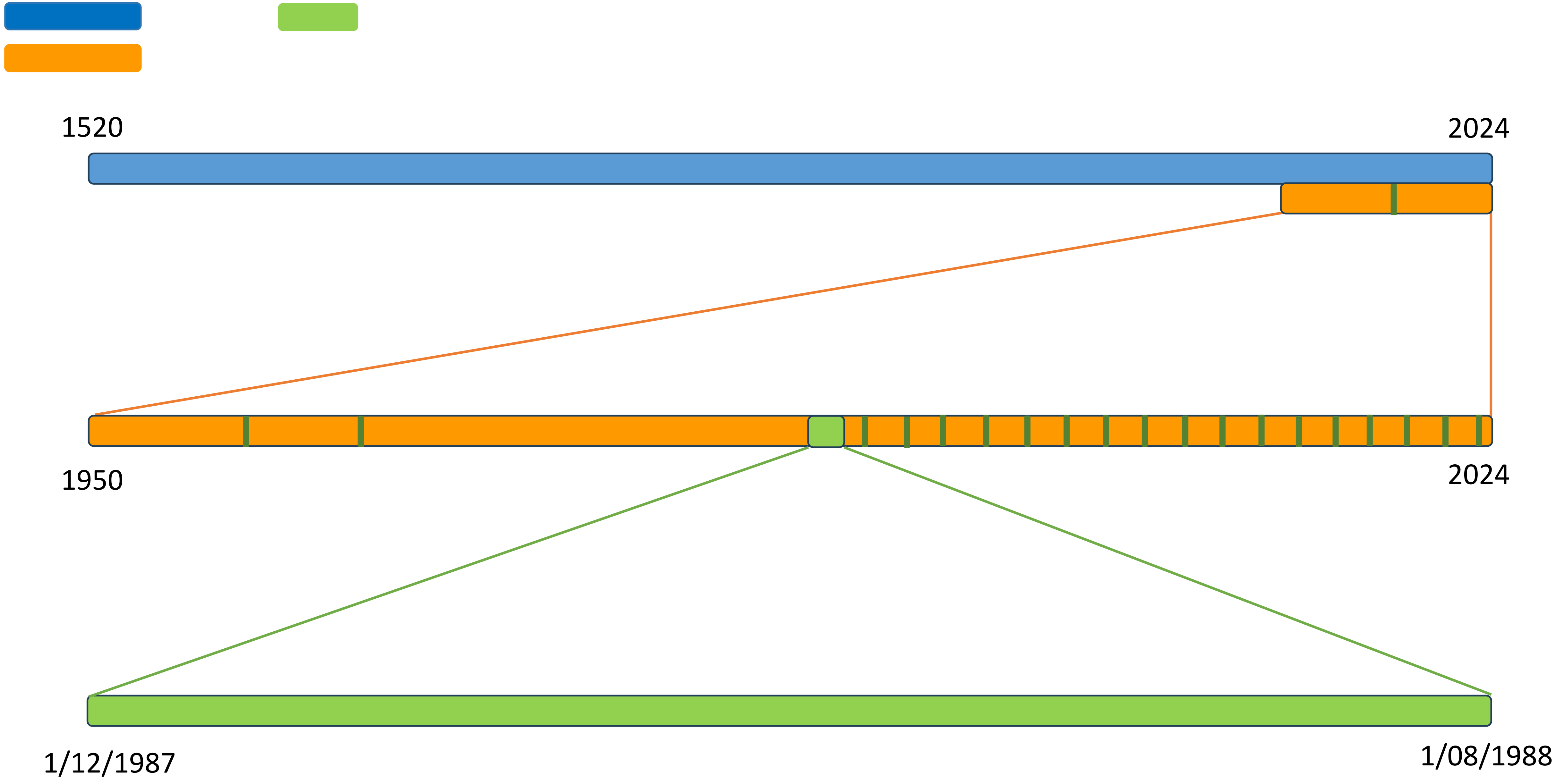
Subject	Age	Estimated Weight of Mussels Consumed†	Domoic Acid in Sample	Estimated Domoic Acid Consumed	Clinical Course‡			
					GI	Memory Loss	Hospitalization	ICU
	yr	*g*	*mg/100 g*	*mg*				
Control	60	35	52	20	–	–	–	–
Patient no.								
1	72	120	52	60	+	–	–	–
2	62	150	45	70	+	+	–	–
3	70	150	52	80	+	–	–	–
4	61	300	31	90	+	–	–	–
5	67	160	68	110	+	–	–	–
6	61	360	31	110	+	–	–	–
7	74	400	68	270	+	+	+	–
8	68	225	128	290	+	+	+	+
9	84	375	76	290	+	+	+	+

*The analysis was limited to persons for whom a sample of uneaten mussels was available.

†The weight of the mussels eaten was estimated when the portion size was unknown.

‡GI denotes gastrointestinal symptoms (vomiting, diarrhea, or abdominal cramps), and ICU admission to the intensive care unit.

Ciguatera versus ASP:



ASP, further advancement:

Harmful Algae 102 (2021) 101975

Contents lists available at ScienceDirect

Harmful Algae

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

Original Article

Marine harmful algal blooms (HABs) in the United States: History, current status and future trends

Donald M. Anderson[a,*], Elizabeth Fensin[b], Christopher J. Gobler[c], Alicia E. Hoeglund[d], Katherine A. Hubbard[d], David M. Kulis[a], Jan H. Landsberg[d], Kathi A. Lefebvre[e], Pieter Provoost[f], Mindy L. Richlen[a], Juliette L. Smith[g], Andrew R. Solow[a], Vera L. Trainer[e]

Fig. 6. Locations where algal toxins were detected in stranded (s) and harvested (h) marine mammals between 2004 and 2013. Red images represent species positive for domoic acid (DA) and purple images represent species positive for saxitoxin (STX). Many species contained both toxins confirming co-exposure. The 13 species that were sampled are listed on the side of the figure in gray (Source: Lefebvre et al., 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ASP, further advancement:

ELSEVIER

Pharmacology & Therapeutics
Volume 227, November 2021, 107865

Public health risks associated with chronic, low-level domoic acid exposure: A review of the evidence

Rebekah Petroff [a], Alicia Hendrix [a], Sara Shum [b], Kimberly S. Grant [a c], Kathi A. Lefebvre [d], Thomas M. Burbacher [a c e]

Table 5: Domoic Acid Human Health Risk Assessments and Suggested Regulatory Limit

Suggested DA limit in shellfish (ppm)	Estimated Seafood Consumption in 1 Meal (g)	Suggested Consumption Limit (mg DA/kg bw)	Citation
19.4	270	0.075	Mariën, 1996
16-24	250-380	0.1	WHO - Toyofuku, 2006
12	200	0.03	LOAEL - Slikker et al., 1998
10	200	0.03	1 in 10,000 risk - Slikker et al., 1998
6.4	200	0.018	Benchmark Dose - Slikker et al., 1998
4.5	400	0.03	EFSA - Alexander et al., 2009
2	135	0.003	Benchmark Dose – Stuchal et al., 2020

ASP, further advancement:

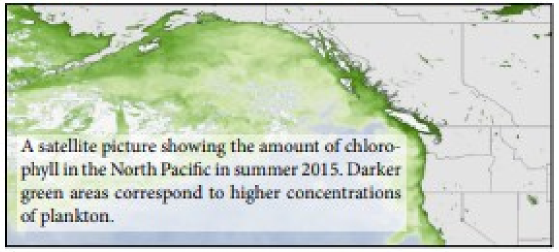
Amnesic Shellfish Poisoning

Pseudo-nitzschia seen unde a microscope. Photo credit: Associated Press

What is Amnesic Shellfish Poisoning?

Amnesic Shellfish Poisoning (ASP) is caused by domoic acid, a toxin produced by marine phytoplankton known as *Pseudo-nitzschia*. When shellfish filter out large amounts of domoic acid and *Pseudo-nitzschia*, they can become contaminated with enough toxin to cause ASP. Humans then get ASP by eating those contaminated shellfish (including clams, mussels, oysters, and crabs).

Symptoms of ASP develop within 48 hours and include vomiting, nausea, and diarrhea. Symptoms for more severe cases include headaches, dizziness, confusion, and permanent short-term memory loss. In rare cases, ASP can lead to coma and death. There is no antidote for domoic acid, but patients with ASP should be taken to a hospital for supportive medical care until the toxin passes through their system.



A satellite picture showing the amount of chlorophyll in the North Pacific in summer 2015. Darker green areas correspond to higher concentrations of plankton.

A sea lion poisoned with domoic acid seizes on a California beach. Thousands of sea lions were sickened during a long-lasting *Pseudo-nitzschia* bloom in 2015.

Deadly Myths

- Shellfish are safe to eat during months containing the letter "r". In November 2015, the entire California crab fishery was shut down due to high levels of domoic acid.
- If the water is clear, there is no danger of shellfish poisoning. Many harmful algal blooms are colorless, including most *Pseudo-nitzschia* blooms. Some shellfish can also retain their toxins for months after a bloom.
- If wildlife has been eating the shellfish, it must be safe. Every animal has a different tolerance to ASP toxins. Do not assume shellfish is safe on the basis of animal observations.
- If shellfish has been tested for Paralytic Shellfish Poisoning (PSP) toxins, it's safe from ASP toxins as well. While multi-species harmful algal blooms are rare, they are becoming increasingly common. ASP and PSP toxins could be present in the same samples.
- Domoic acid can be cooked or frozen out of shellfish. This toxin is heat stable and cannot be removed.

How can I avoid ASP?

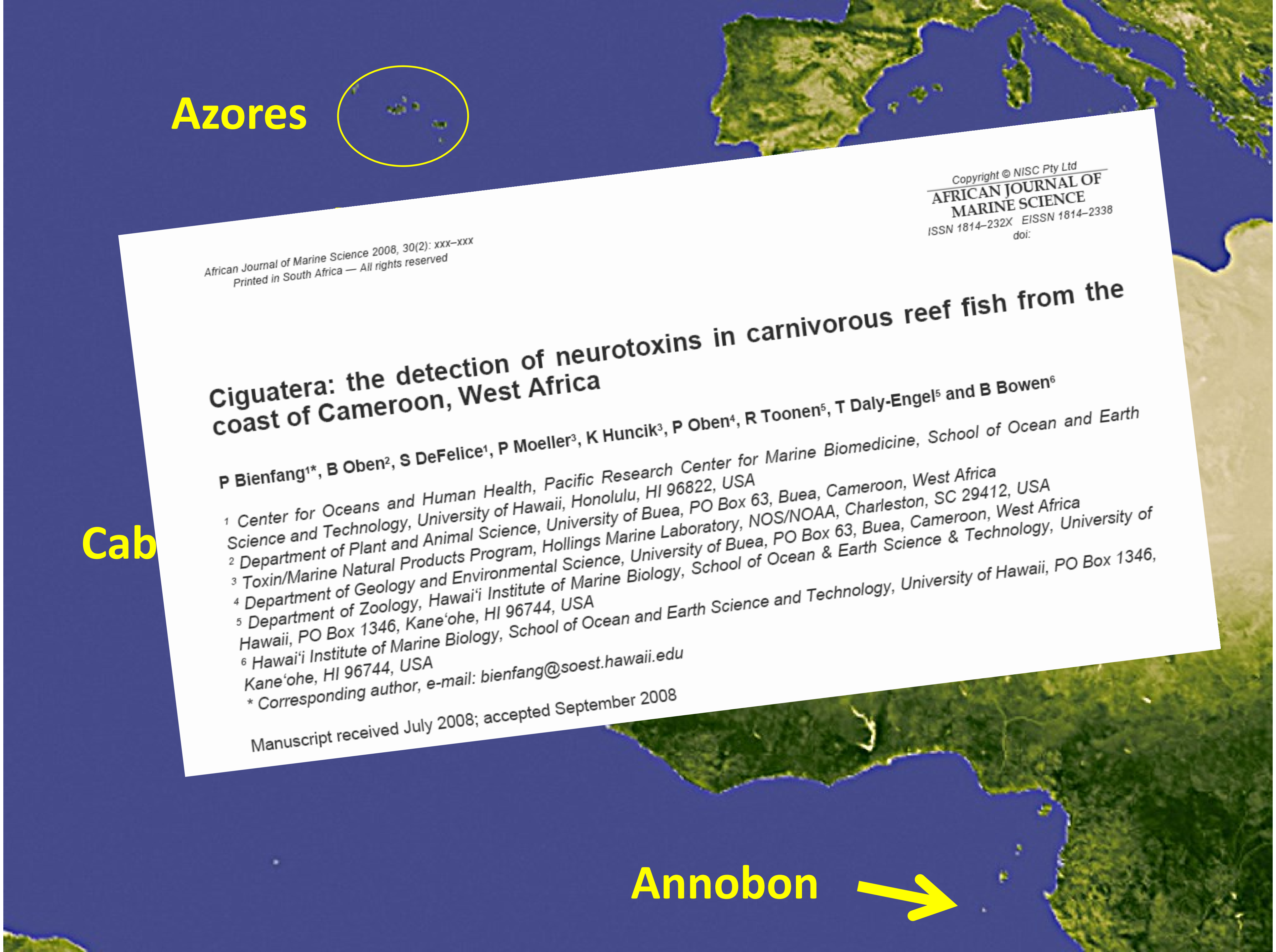
- Get a sample of your shellfish tested for ASP toxins at the Sitka Tribe's Environmental Lab (contact information below). This is the best way to ensure that you're consuming safe shellfish.
- Clean your crabs. Domoic acid accumulates in the crab viscera, or "butter", so crab guts should be thoroughly cleaned out BEFORE cooking.
- Check out seator.org for the latest info on which plankton have been common lately. NOTE: while this is an excellent first step to becoming a more informed shellfish harvester, not seeing current harmful algal blooms does not guarantee shellfish safety.

SEATOR Culture Science Efficiency Southeast Alaska Tribal Ocean Research

Testing Information

Contact Michael Jamros, the Sitka Tribe of Alaska's Envirnomental Lab Manager, for information on testing availability and harvesting protocols. Phone: 747-7356 Email: seator@sitkatribe-nsn.gov

Ciguatera:



African Journal of Marine Science 2008, 30(2): xxx–xxx

AFRICAN JOURNAL OF MARINE SCIENCE
ISSN 1814–232X EISSN 1814–2338
doi:

Ciguatera: the detection of neurotoxins in carnivorous reef fish from the coast of Cameroon, West Africa

P Bienfang[1]*, B Oben[2], S DeFelice[1], P Moeller[3], K Huncik[3], P Oben[4], R Toonen[5], T Daly-Engel[5] and B Bowen[6]

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[3] Toxin/Marine Natural Products Program, Hollings Marine Laboratory, NOS/NOAA, Charleston, SC 29412, USA
[4] Department of Geology and Environmental Science, University of Buea, PO Box 63, Buea, Cameroon, West Africa
[5] Department of Zoology, Hawai'i Institute of Marine Biology, School of Ocean & Earth Science & Technology, University of Hawaii, PO Box 1346, Kane'ohe, HI 96744, USA
[6] Hawai'i Institute of Marine Biology, School of Ocean and Earth Science and Technology, University of Hawaii, PO Box 1346, Kane'ohe, HI 96744, USA
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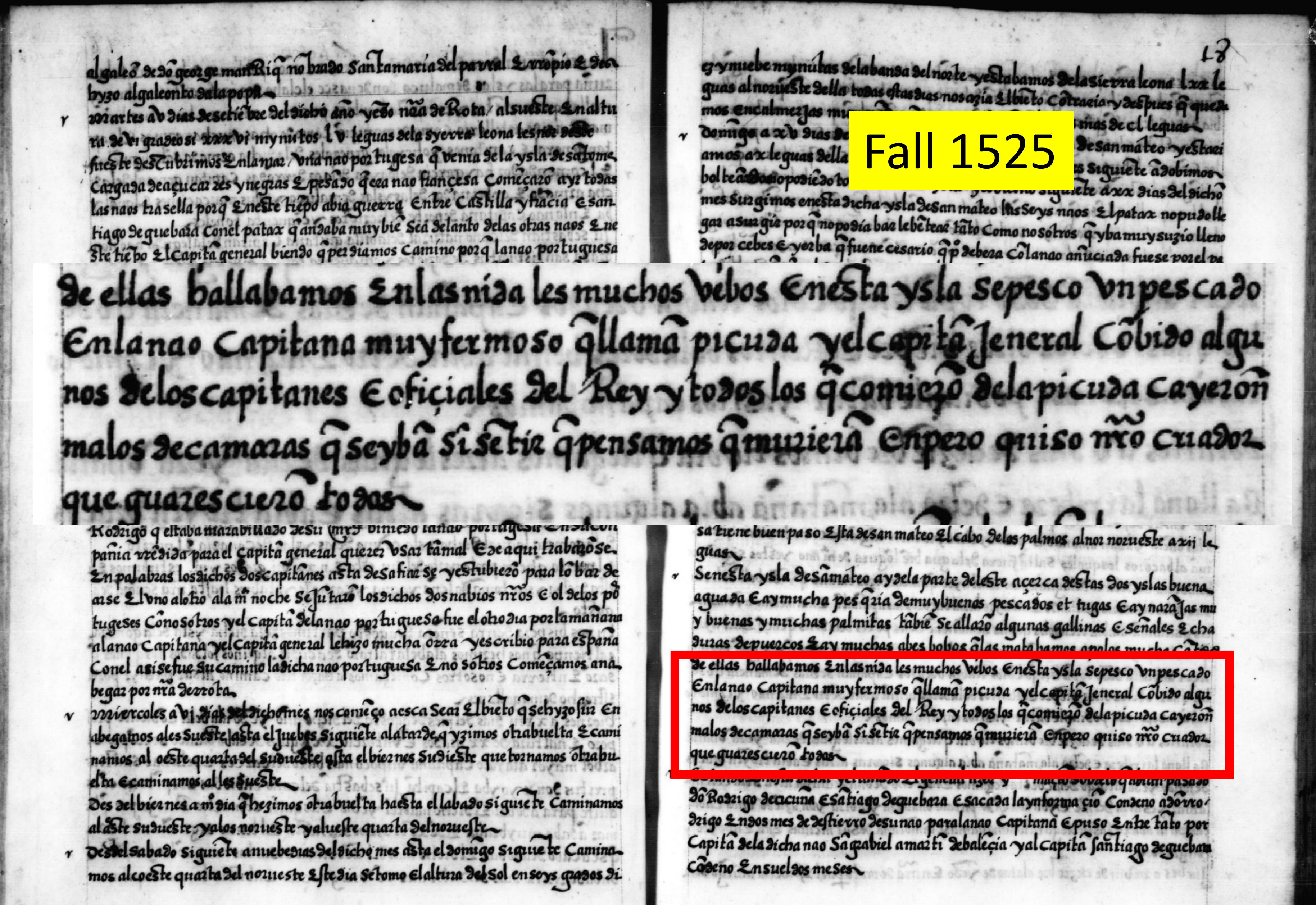
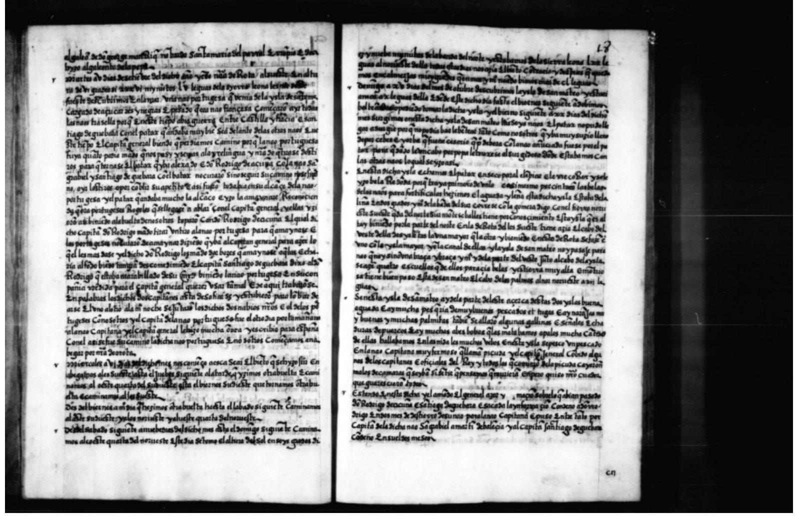
Manuscript received July 2008; accepted September 2008



Urdaneta, 1580



Probably, the first report of a ciguatera case dates from 1525, from the Gulf of Guinea and affected the crew of a Spanish vessel (second trip to the Pacific through the Magallanes Strait).

"En esta ysla se pesco un pescado en la nao capitana muy fermoso q^ llama^ picuda y el capita^ Jeneral côbido algunos de los capitanes e oficiales del Rey y todos los q^ comiero^ de la picuda cayeron^ malos de camaras q^ seyba^ sî sêtir q^ pensamos q^ muriera^ enpero quiso n^tro criador que guaresciero^ todos"

"On this island a splendid fish was fished on the captain boat that is called barracuda and the General Captain invited some of the captains and officials of the King and all who ate part of the barracuda had diarrhea, were losing sense and we thought they were dying but our Creator decided that all should recover"

Urdaneta, 1580

(Santi Fraga)

Bulletin de l'I. F. A. N.
T. XVIII, sér. A, n° 2, 1956.

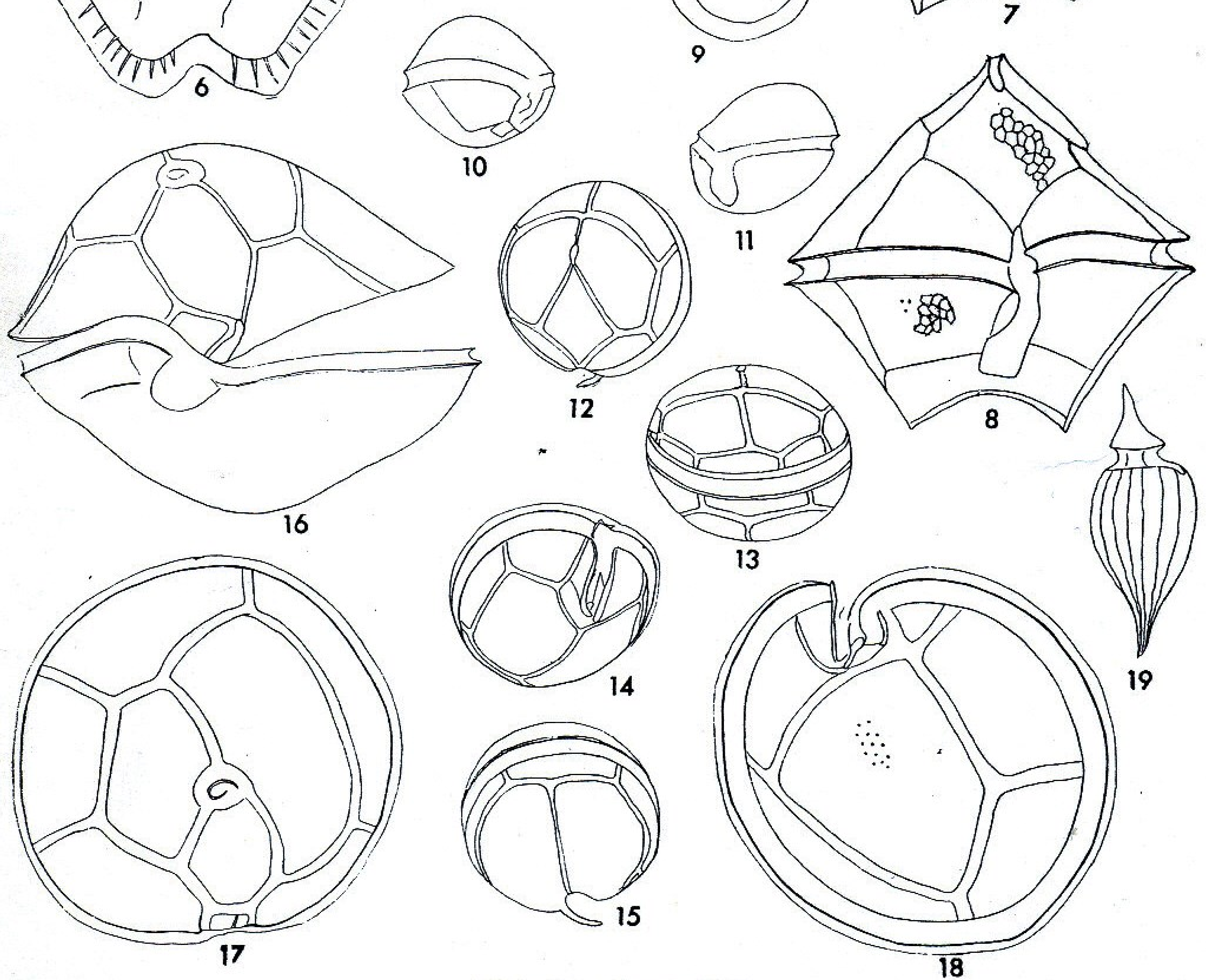
1948

Contribution à l'étude du microplancton de Dakar et des régions maritimes voisines

par Estela de SOUSA e SILVA.

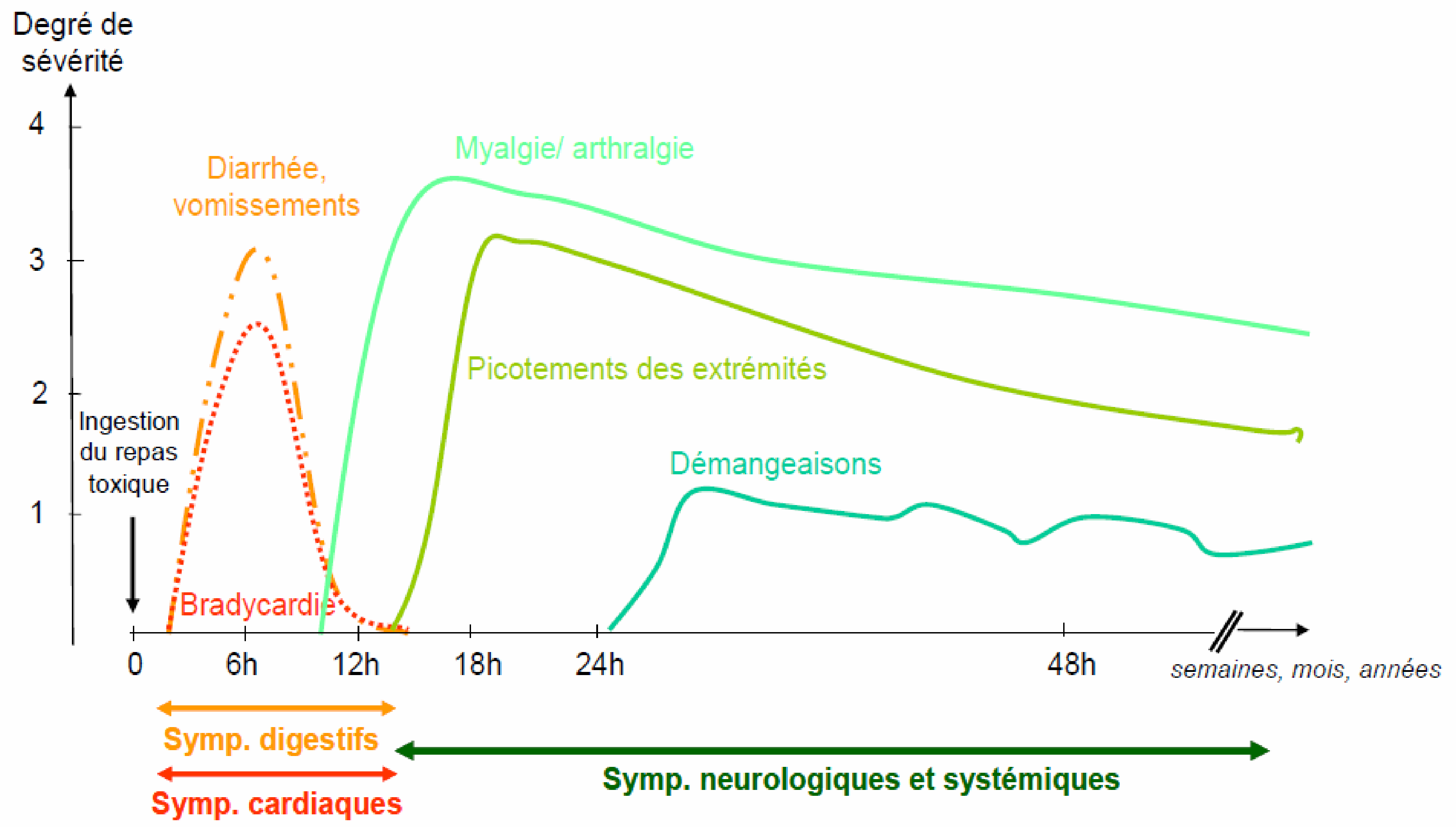
Dans cette note nous présentons les résultats obtenus par l'analyse de 73 échantillons de plancton, faisant partie des collections de l'Ifan, récoltés sur les côtes du Sénégal, Cap Vert, Côte-d'Ivoire, qui nous ont été aimablement communiqués par M. J. Cadenat, chef de la Section Biologie Marine de l'Institut Français d'Afrique Noire, à qui nous adressons nos vifs remerciements.

Notre attention a été portée, tout spécialement, sur les Diato-



(*Voir légende page 359.*)

Chronology of appearance of the principal symptoms of Ciguatera



Chinain, Gatti, Darius, 2013
Bulletin de Veille Sanitaire

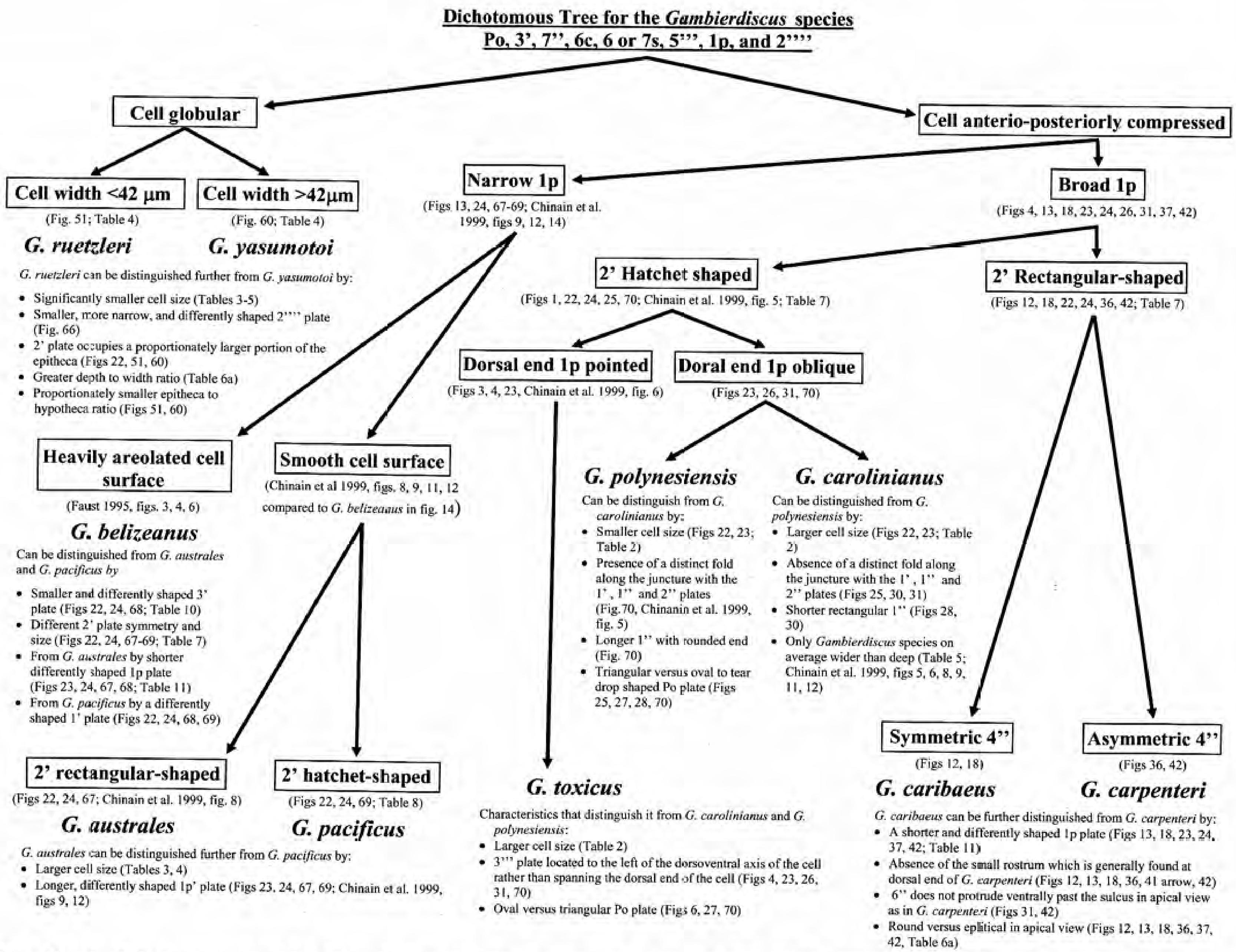


Fig. 10. A dichotomous tree detailing the morphometrics (cell size, shape and plate structure) used to distinguish the various *Gambierdiscus* species.

Gamberdiscus spp Macaronesia:

Harmful Algae 11 (2011) 10–22

Contents lists available at ScienceDirect

Harmful Algae

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

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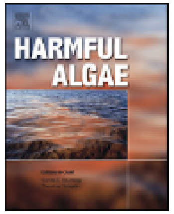


Gambierdiscus excentricus sp. nov. (Dinophyceae), a benthic toxic dinoflagellate from the Canary Islands (NE Atlantic Ocean)

Santiago Fraga[a,*], Francisco Rodríguez[a], Amandine Caillaud[b], Jorge Diogène[b], Nicolás Raho[c], Manuel Zapata[d]

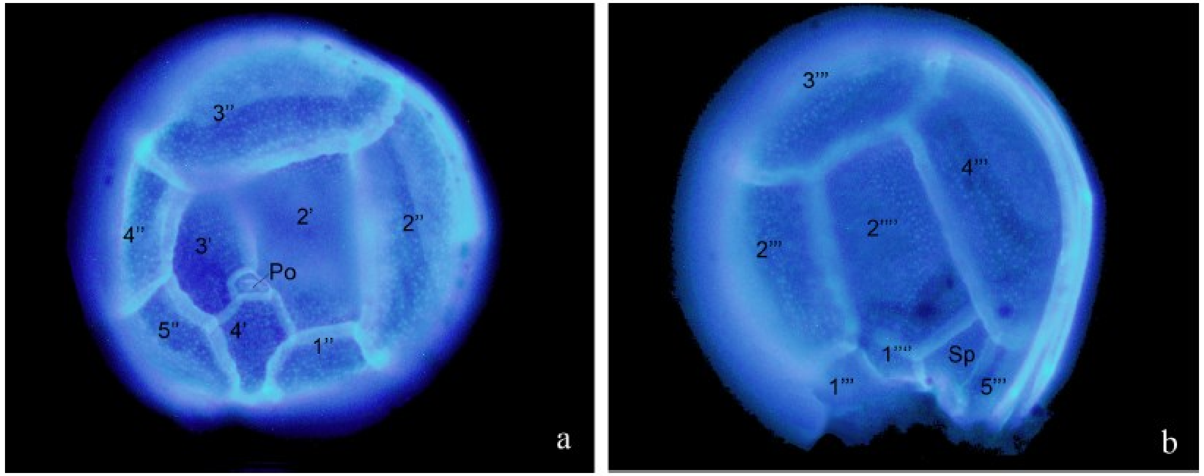


Fig. 1. Epitheca (a) and hypotheca (b) of Gambierdiscus australes cells stained with Calcofluor White.

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: cigua-

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

- First identification of ciguatoxins in shark (*Carcharhinus leucas*, bull shark – Madagascar)
- New CTX analogues: I-CTX-5 & I-CTX-6
- First identification of gambieric acid in fish.

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Identification of ciguatoxins in a shark involved in a fatal food poisoning in the Indian Ocean

Received: 10 March 2017
Accepted: 20 July 2017
Published online: 15 August 2017

Jorge Diogène[1], Laia Reverté[1], Maria Rambla-Alegre[1], Vanessa del Río[1], Pablo de la Iglesia[1], Mònica Campàs[1], Oscar Palacios[2], Cintia Flores[2], Josep Caixach[2], Christian Ralijaona[3], Iony Razanajatovo[4], Agathe Pirog[5], Hélène Magalon[5], Nathalie Arnich[6] & Jean Turquet[7]

- **Occurred in November 2013 in Fenerive-Est, Madagascar**
- **94 people poisoned, 11 of whom died**
- **Ciguatera symptoms : neurological and digestive signs.**
- **After eating the flesh, the liver, the head, and part of the viscera of a bull shark (*Carcharinus leucas*).**

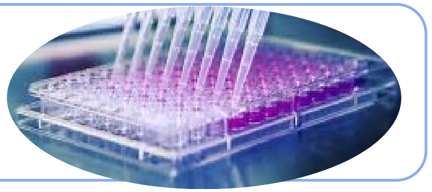
Rabenjarison, F. et al. Ciguatera poisoning after consumption of shark in Fenerive-Est: epidemiological and clinical aspects and laboratory results. Revue d’Anesthésie-réanimation, Médecine d’Urgence et Toxicologie 8 (1), 9–12 (2016). ISSN 2225-5257. Accessible online at http://rarmu.org/publications/8(1)/full_text/8(1)9-12.pdf.

- Gather epidemiologic information of the poisoning event
- Confirm species of shark and individual

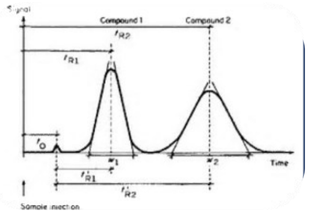
- Processing shark tissues: toxin extraction

- Analysis of shark tissues by MBA
- List symptoms in mice

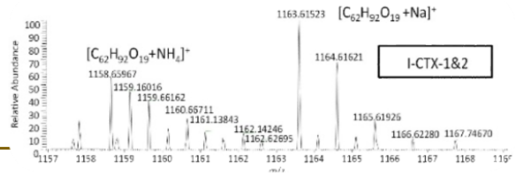
- CTX screening by CBA



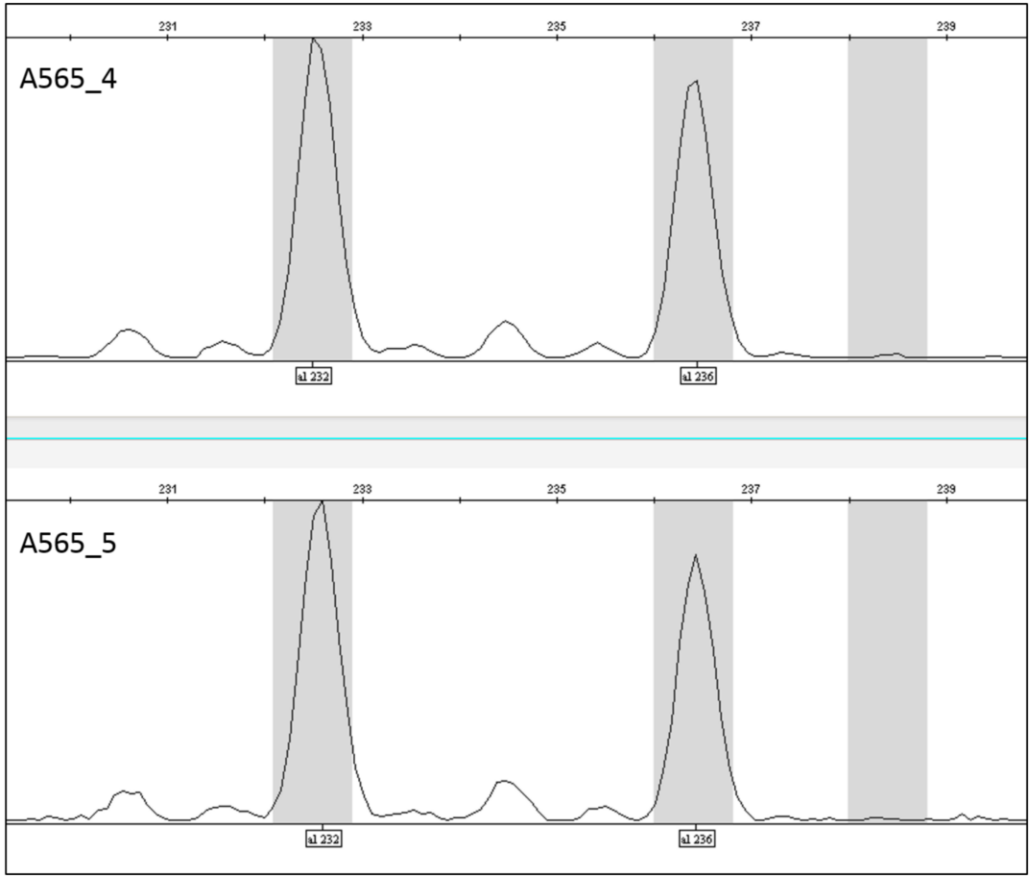
- Stomach chromatographic fractionation



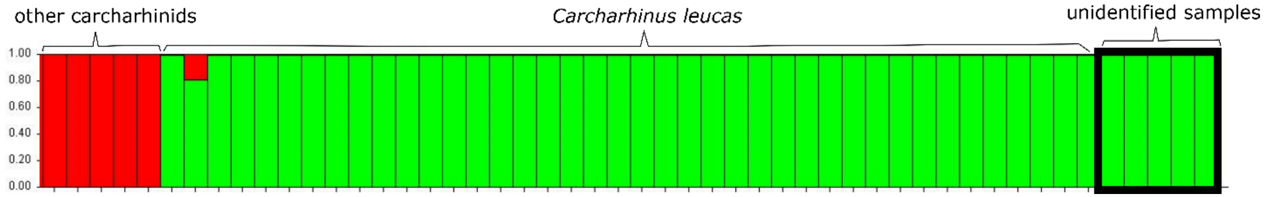
- Confirmation and quantification of CTXs by LC-HRMS



Species and individual confirmation:



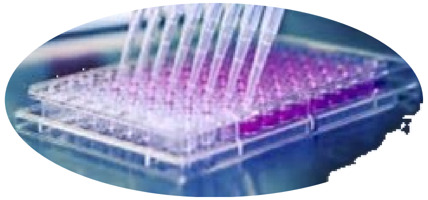
Example of a chromatogram for samples A565_4 and A565_5 for the microsatellite locus Cl11. Black peaks represent alleles with allele sizes indicated by the labels.

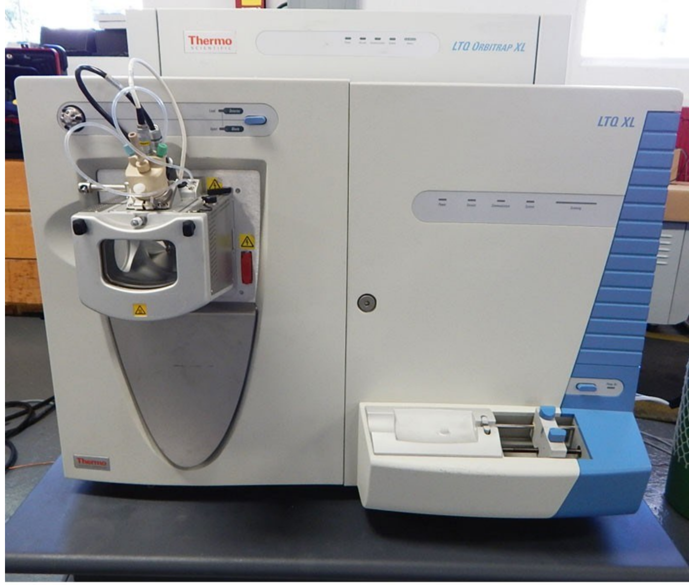


Assignment test (STRUCTURE software) for shark species identification. Each bar on the x-axis represents one individual and the y-axis represents the probability to belong to one or another cluster. The multilocus genotypes of the unidentified shark samples (presumed to be bull shark) were used in assignment with other bull shark individuals (from Pirog et al. 2015) and other carcharinid species that successfully amplified the loci used (*Carcharhinus obscurus* or *Carcharhinus plumbeus*). We found two clusters: the red one corresponds to other shark species and the green one corresponds to bull shark individuals. The unidentified samples clustered with the bull shark cluster.

Same individual of *Carcharhinus leucas*

CTXs quantification:

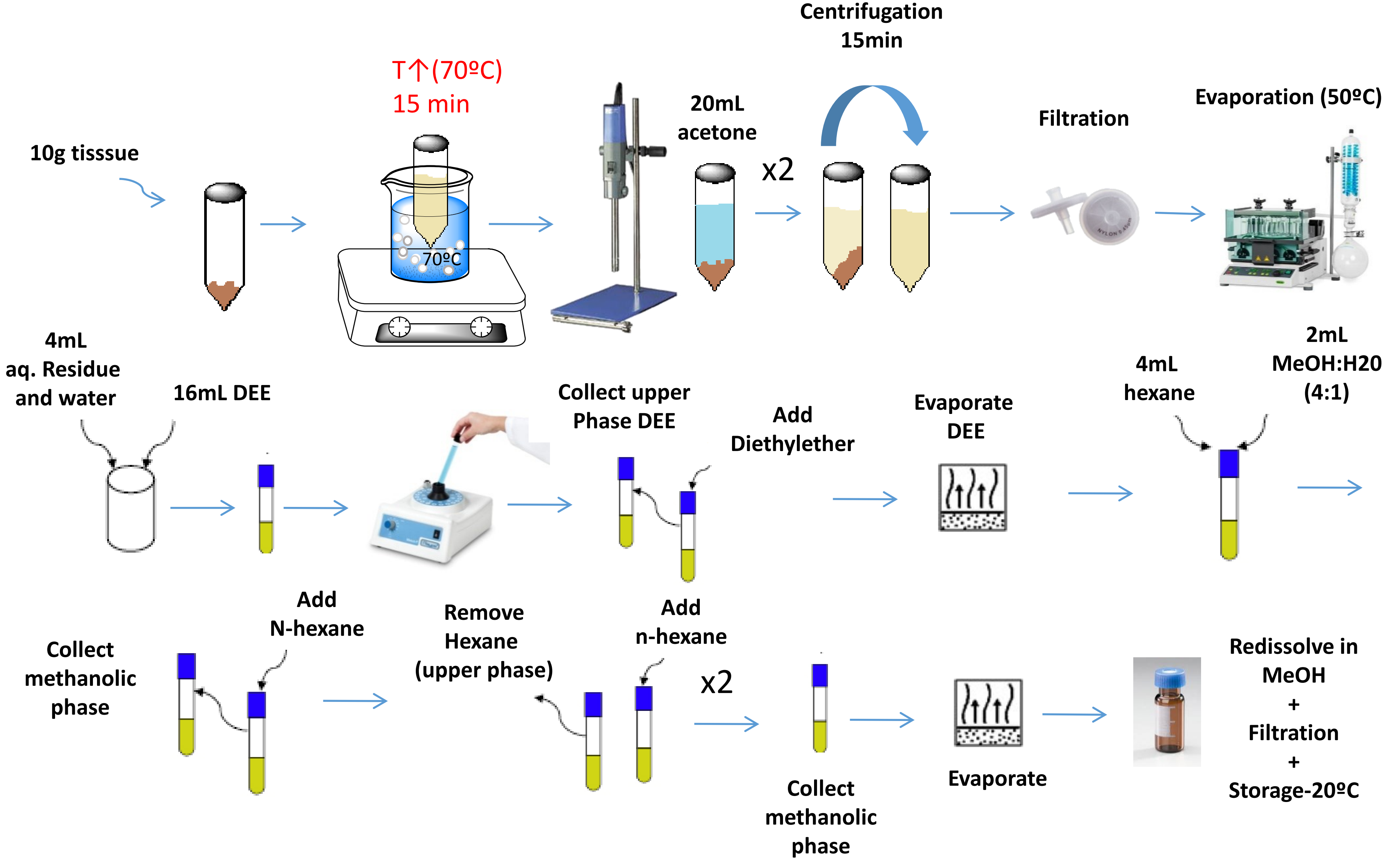




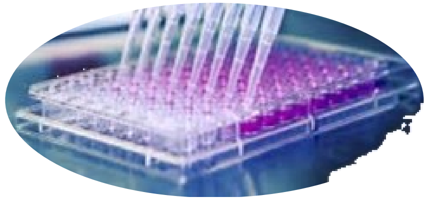
Crude extract	MBA (µg P-CTX-1 equiv./kg tissue)	CBA (µg P-CTX-1 equiv./kg tissue)	LC-ESI-HRMS (µg P-CTX-1 equiv./kg tissue)		
			I-CTX-1&2	I-CTX-3&4	Σ I-CTXs
flesh	n.q.	0.06	n.d.	n.d.	n.d.
stomach	83	92.78	6.54	9.74	16.28
fin 1	-	0.12	-	-	-
fin 2	-	0.79	n.d.	n.d.	n.d.
fin 3	-	0.17	-	-	-

Concentration of P-CTX-1 equiv./kg tissue in crude stomach, flesh and fin extracts as determined by mouse bio-assay (MBA), Neuro-2a cell-based assay (CBA) and liquid chromatography coupled to high resolution mass spectrometry (LC-ESI-HRMS).

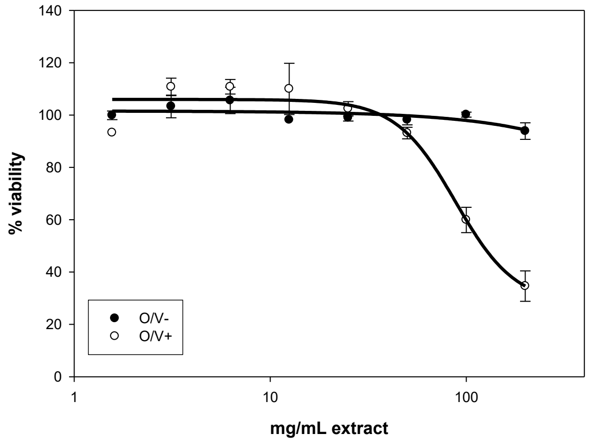
CTXs extraction:



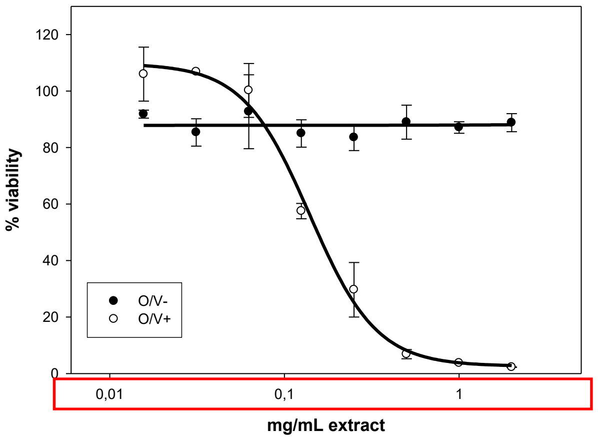
CBAs toxicity evaluation in crude extracts:



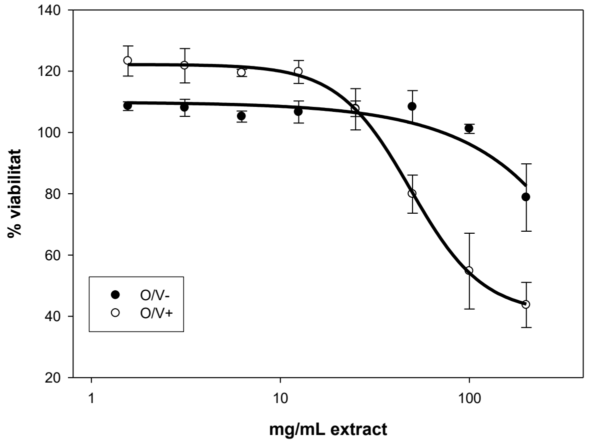
Flesh



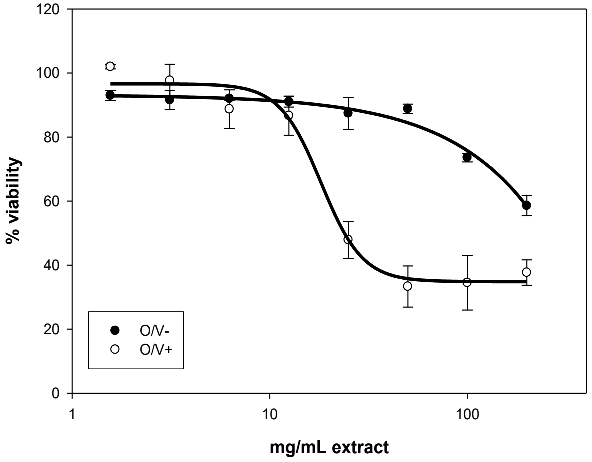
Stomach



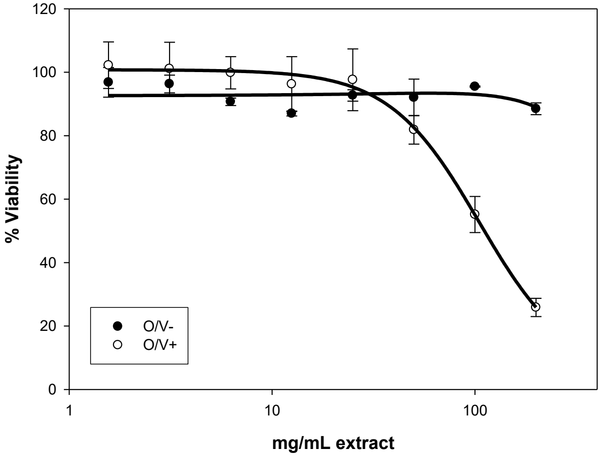
Fin 1



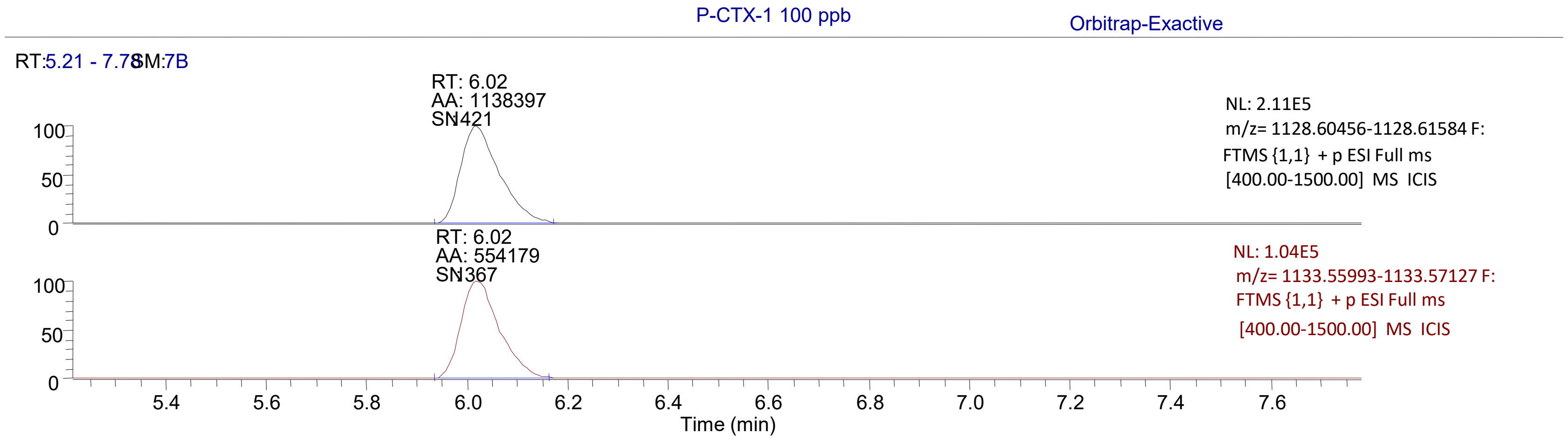
Fin 2

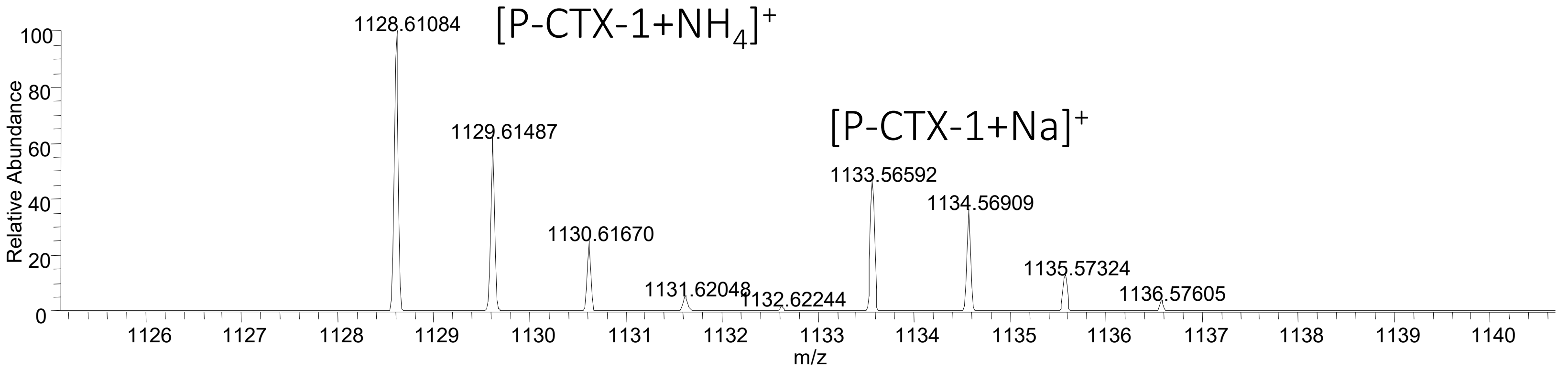


Fin 3



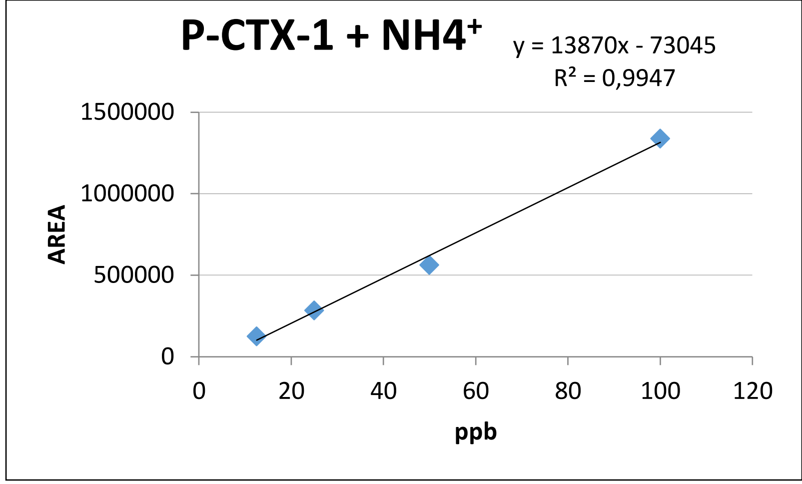
Standard CTX-1B:

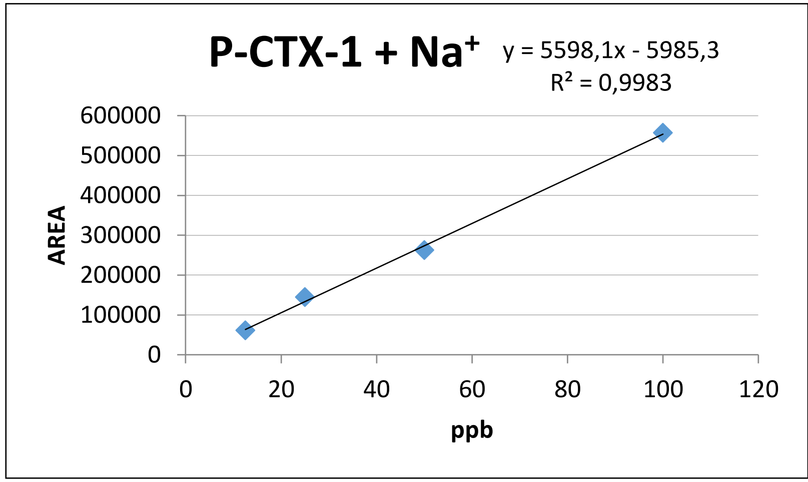


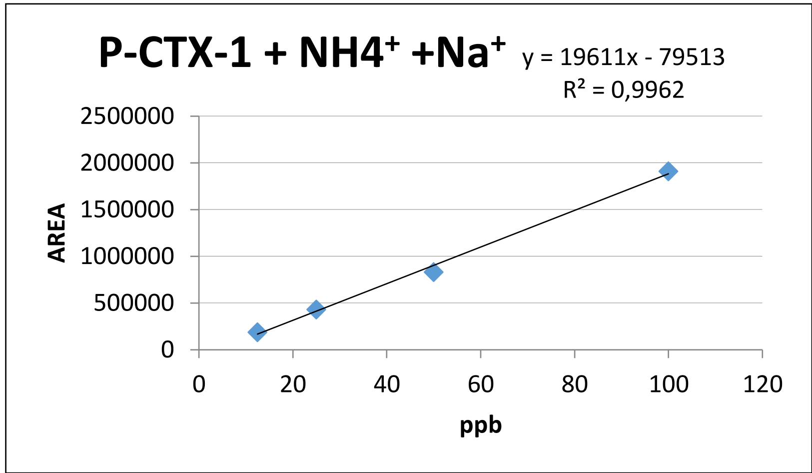


- LC: Hypersil Gold, 1.9um, 50x2.1 mm
- 200 ul/min
- Orbitrap Exactive-HCD
- Resolució 50000 (2Hz)
- Full scan: 400-1500Da
- ESI +

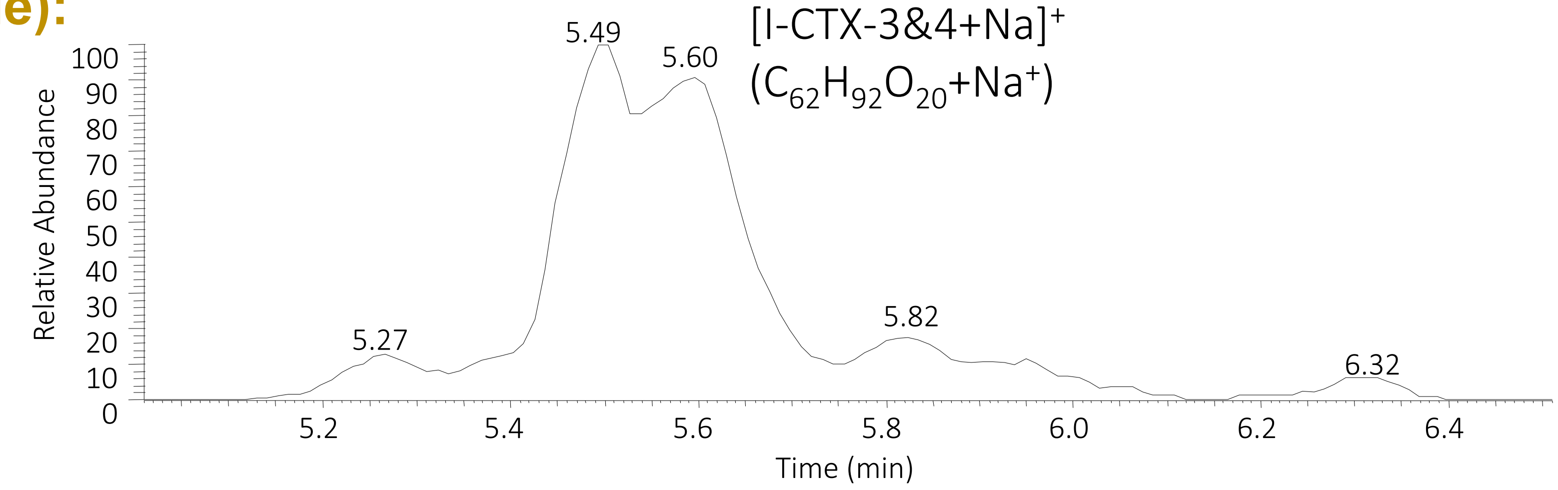
Linearity range **P-CTX-1**
12.5-25-50-100 ppb
-Quantification:
P-CTX-1+NH4$^+$+Na$^+$

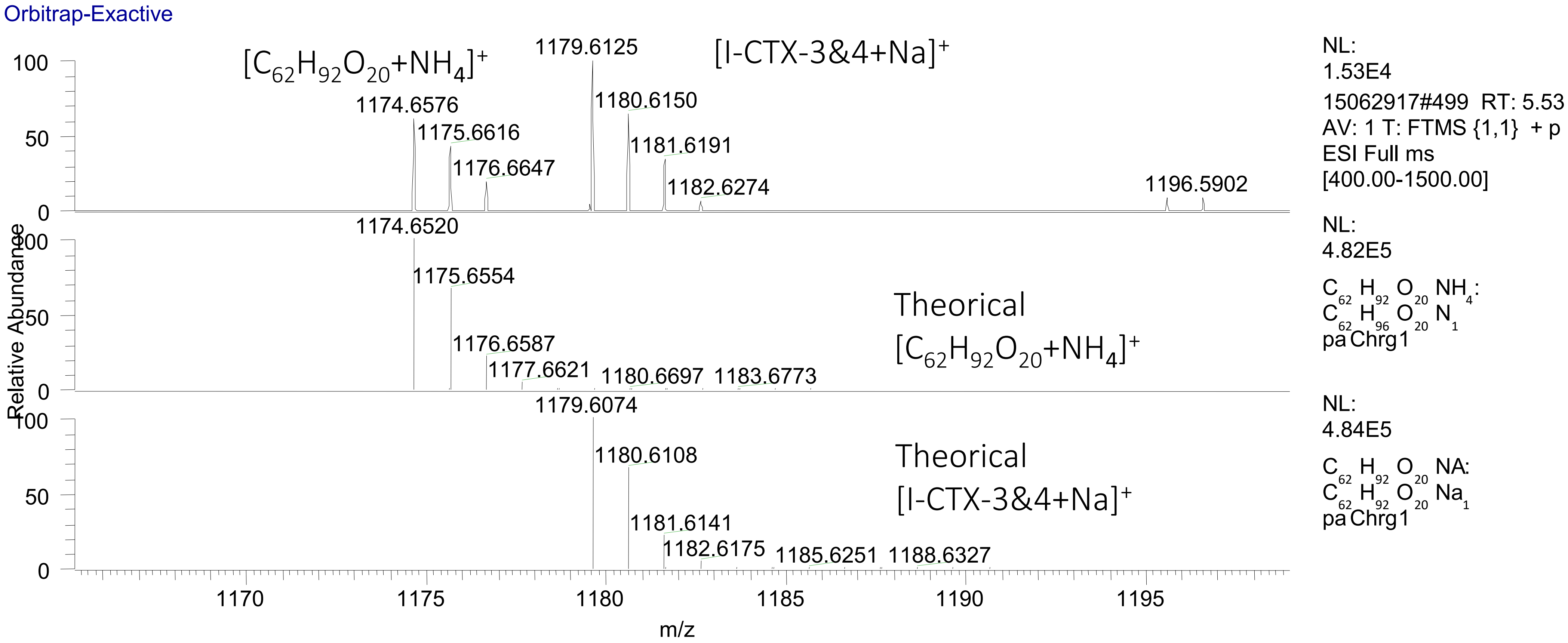






LC-HRMS I-CTX-3&4 (Stomach, crude):





Dissolved CTXs:

Harmful Algae 10 (2011) 433–446

Contents lists available at ScienceDirect

Harmful Algae

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

Monitoring of dissolved ciguatoxin and maitotoxin using solid-phase adsorption toxin tracking devices: Application to *Gambierdiscus pacificus* in culture

Amandine Caillaud [a], Pablo de la Iglesia [a], Esther Barber [a], Helena Eixarch [a], Normawaty Mohammad-Noor [b], T. Yasumoto [c], Jorge Diogène [a,*]

[a] IRTA, Ctra. Poble Nou, km. 5.5, 43540 Sant Carles de la Ràpita, Spain
[b] Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[c] Japan Food Research Laboratory, Tama Laboratory, 6-11-10 Nagayama, Tama-shi, Tokyo 206-0025, Japan

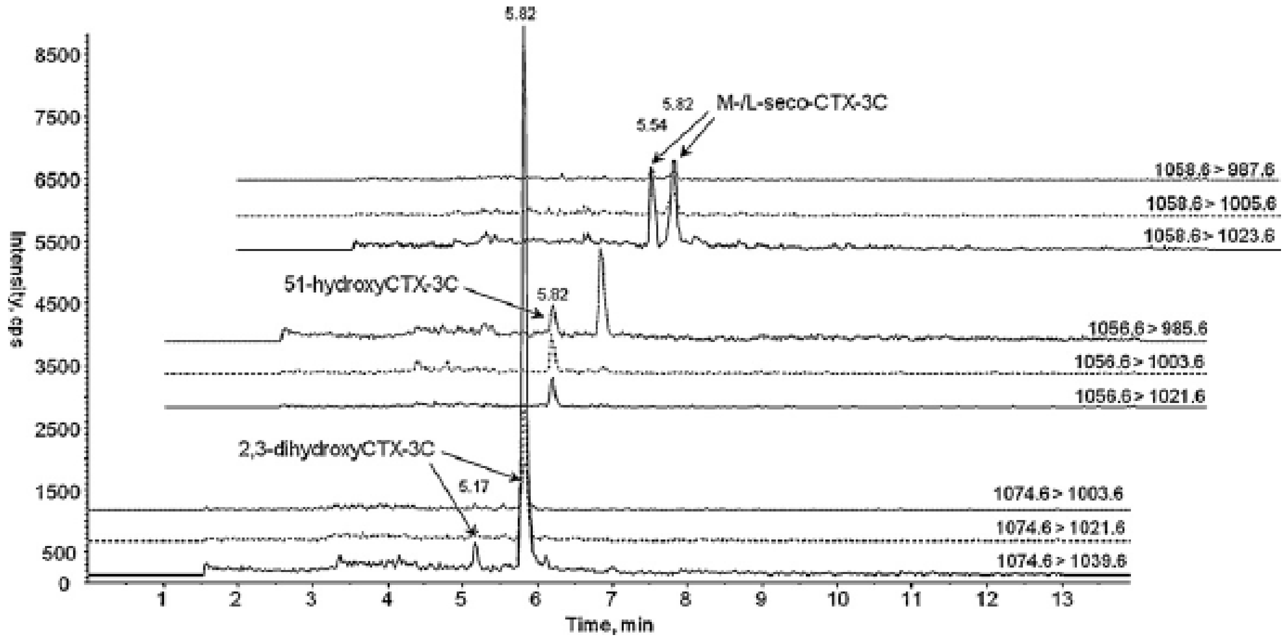


Fig. 8. LC–MS/MS analysis of a resin extract obtained after exposure to a culture of *G. pacificus* (strain G10DC) over the period T44–T47 (senescence phase). Extracted ions chromatograms has been overlapped with 3 MRM pairs for each congener for the tentatively identified 2,3-dihydroxyCTX-3C, 51-hydroxyCTX-3C and M-/L-seco-CTX-3C. Chromatographic and MS/MS detection conditions were applied as described for LC–MS/MS analysis (Section 2.9).

Dissolved CTXs:

Harmful Algae 71 (2018) 40–49

Contents lists available at ScienceDirect

Harmful Algae

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

Application of solid phase adsorption toxin tracking (SPATT) devices for the field detection of *Gambierdiscus* toxins

Mélanie Roué[a,*], Hélène Taiana Darius[b], Jérôme Viallon[b], André Ung[b], Clémence Gatti[b], D. Tim Harwood[c], Mireille Chinain[b]

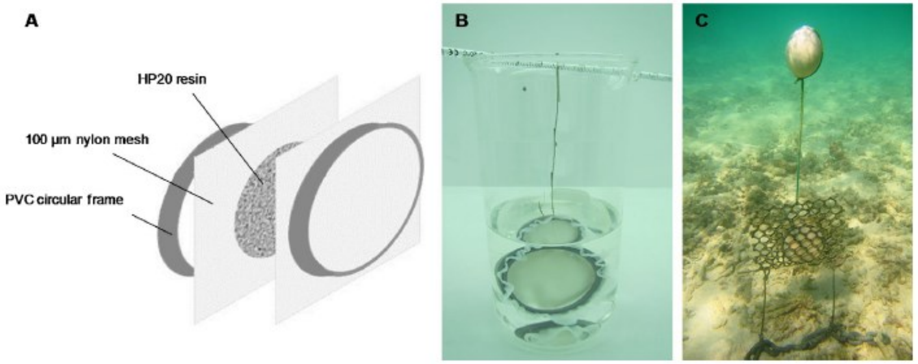


Fig. 1. Passive sampler devices. (A) SPATT bag assembly; (B) SPATT bag exposed to TB92–*G. polynesiensis* cells; (C) SPATT bag deployed in a French Polynesian lagoon.

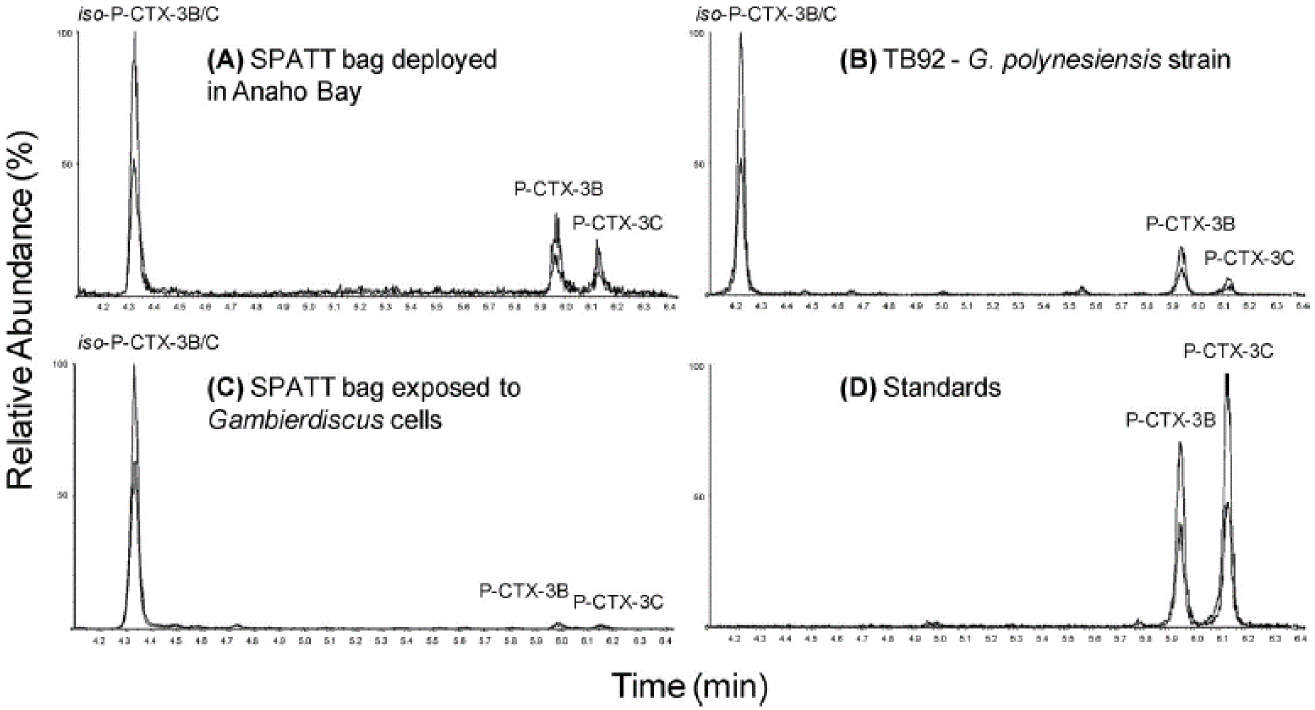
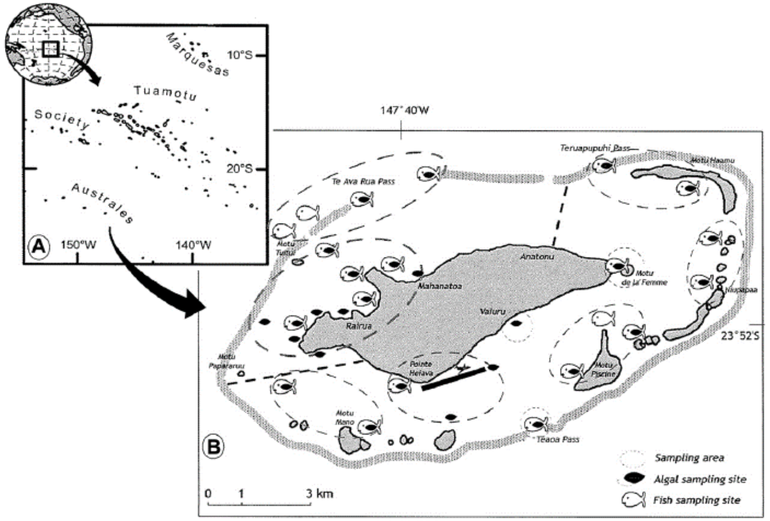


Fig. 5. LC–MS/MS chromatograms of methanolic extracts of (A) a SPATT bag deployed into Anaho Bay (Nuku Hiva Island); (B) TB92 – *G. polynesiensis* cells; (C) a SPATT bag exposed to TB92 – *G. polynesiensis* cells; and of (D) P-CTX–3 B and P-CTX-3C standards. Shown in each panel are overlayed chromatograms generated from the quantification (1023.6 > 155.1) and confirmation (1023.6 > 125.1) transitions, allowing a comparison of the ion ratios observed in samples and standards.

Monitoring for *Gambierdiscus* spp:



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Contents lists available at ScienceDirect

Toxicon

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

Ciguatera risk management in French Polynesia: The case study of Raivavae Island (Australes Archipelago)

Mireille Chinain [a,*], H. Taiana Darius [a], André Ung [a], Mote Tchou Fouc [a], Taina Revel [a], Philippe Cruchet [a], Serge Pauillac [d], Dominique Laurent [b,c]

International/National/Regional approaches:

Pacific Tahiti

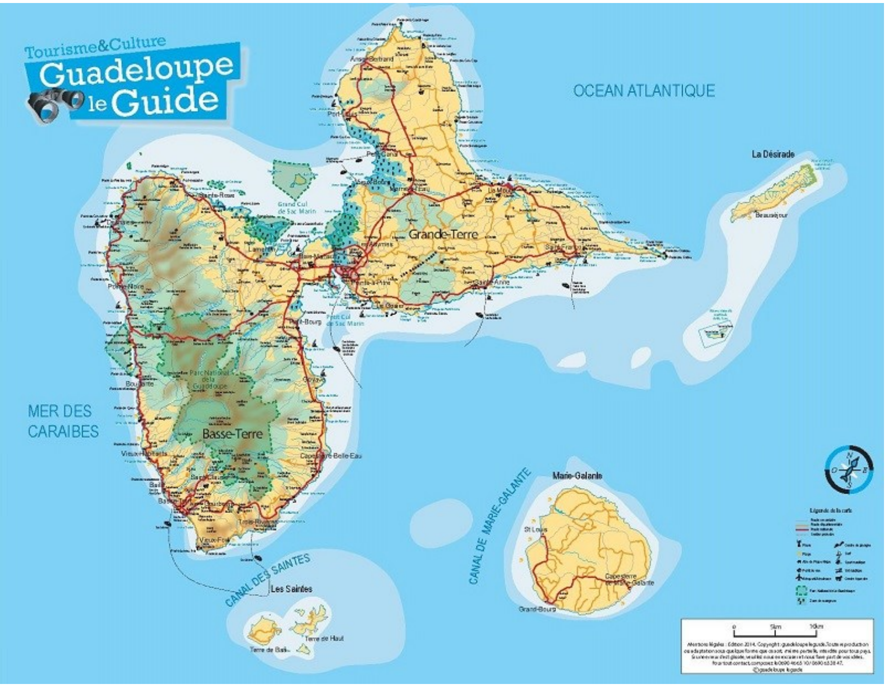
No official methods

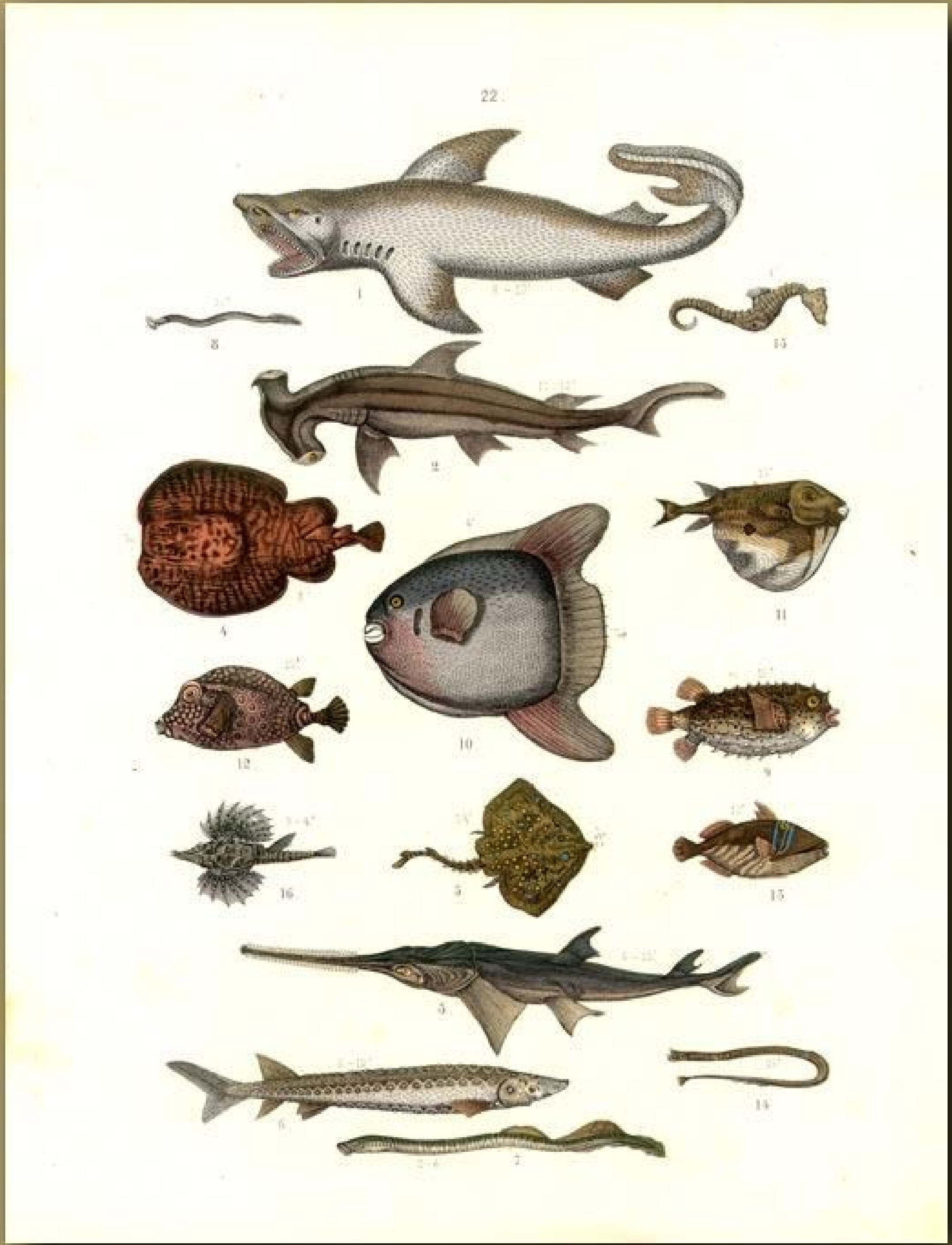
No maximum permitted levels

CBA-N2a and Receptor Binding Assay (RBA), and are presently working on the implementation of the fluorescent RBA

Existing monitoring programmes for CTXs in fish and *Gambierdiscus* spp. on a contract basis (not permanent) : e.g Raivavae Island, case recording and incidence, community interviews, fish, *Gambierdiscus* spp. densities, toxin evaluation, risk ranking.

CTXs, Guadeloupe, Caribbean:





CTXs, Guadeloupe, Caribbean:

- **2010-2012 35 Ciguatera Fish poisoning events affecting 87 individuals.**
- **For 12 of these events the meal remnants or uncooked fish was available**
- **Epidemiological data were recorded for each patient**
- **In some cases, the estimated amount of food was available**

The Neuro-2a CBA applied to risk characterization: LOAEL for CTXs in an epidemiological study

ELSEVIER

Contents lists available at ScienceDirect

Environmental Research

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

Contribution to the risk characterization of ciguatoxins: LOAEL estimated from eight ciguatera fish poisoning events in Guadeloupe (French West Indies)

Virginie Hossen [a], Lucia Soliño [b], Patricia Leroy [a], Eric David [c], Pierre Velge [d], Sylviane Dragacci [a,*], Sophie Krys [a], Harold Flores Quintana [e], Jorge Diogène [b]

[a] *Université Paris-Est, ANSES-Laboratory for Food Safety, National Reference Laboratory for the Control of Marine biotoxins, 14 rue Pierre et Marie Curie, 94701 Maisons-Alfort, France*

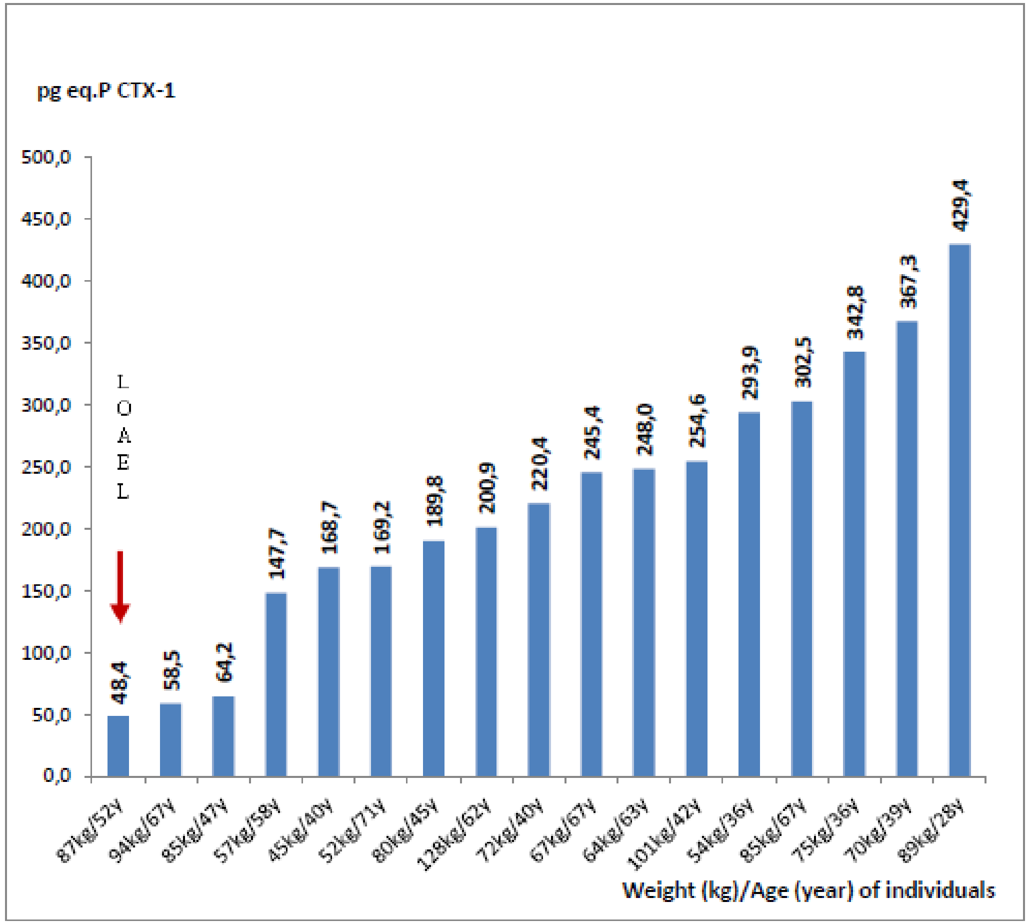
[b] *Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Ctra. Poble Nou km 5.5, Sant Carles de la Rapita, Spain*

[c] *Ministry of Agriculture, Direction de l'Alimentation de l'Agriculture et de la Forêt de Guadeloupe, Abymes, France*

[d] *Ministry of Agriculture, General Directorate for Food, Paris, France*

[e] *U.S. Food and Drug Administration (FDA), Division of Seafood Science and Technology, Gulf Coast Seafood Laboratory, 1 Iberville Drive, Dauphin Island, AL 36528, USA*

Figure 2: Toxin intakes in a series of CFP events in Guadeloupe, 2010-2012, and the estimation of the LOAEL. Toxin intakes are ranked from lowest to highest estimated values expressed in pg P-CTX-1 eq./kg bw from the 17 individual cases fully documented.



The Neuro-2a CBA applied to risk characterization: LOAEL for CTXs in an epidemiological study

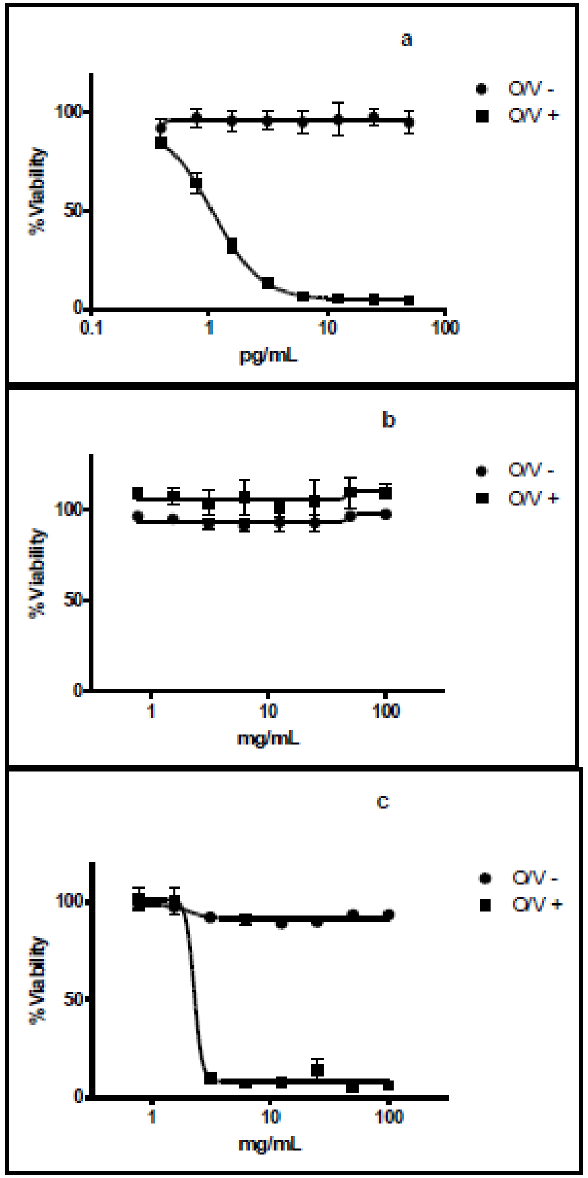


Figure 1: Evaluation of cytotoxicity of fish extracts and P-CTX-1 standard by Neuro-2A cells: a) P-CTX-1 standard dose-response curve; b) negative control of a jack fish showing no toxicity in the absence (OV-) or presence (OV+) of ouabaine and veratridine; c) dose-response curve of *Lutjanus* sp. extract from event 10. Fish extract concentration is expressed in mg/mL of tissue equivalents.

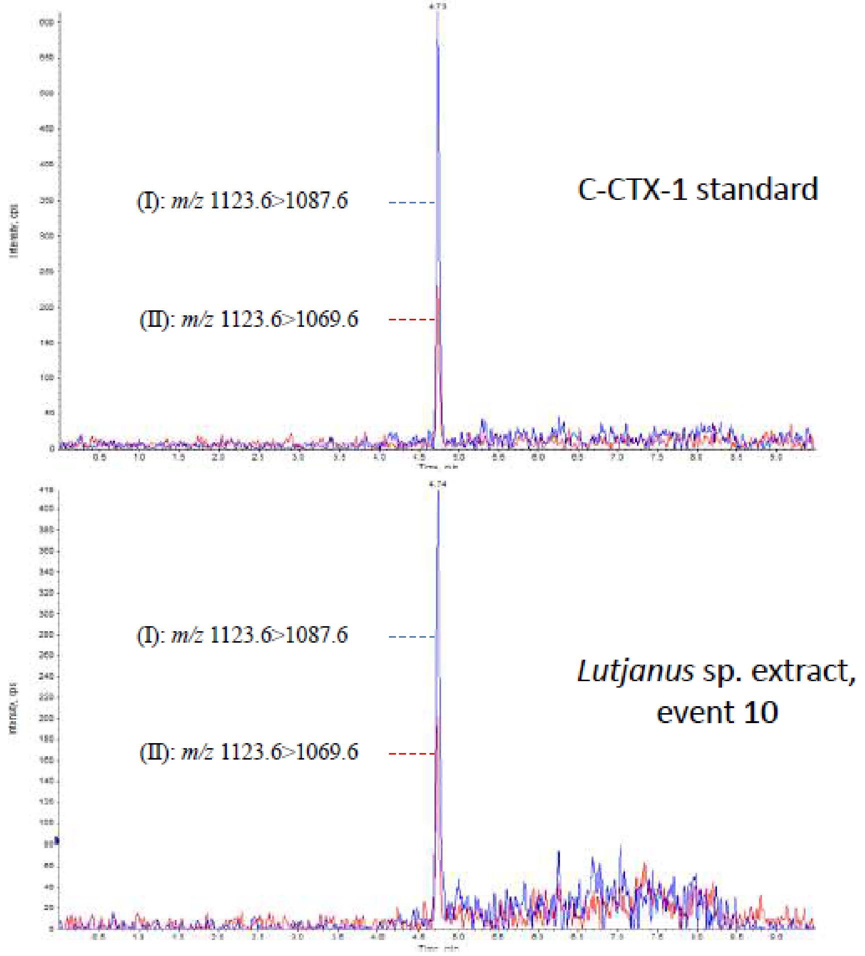
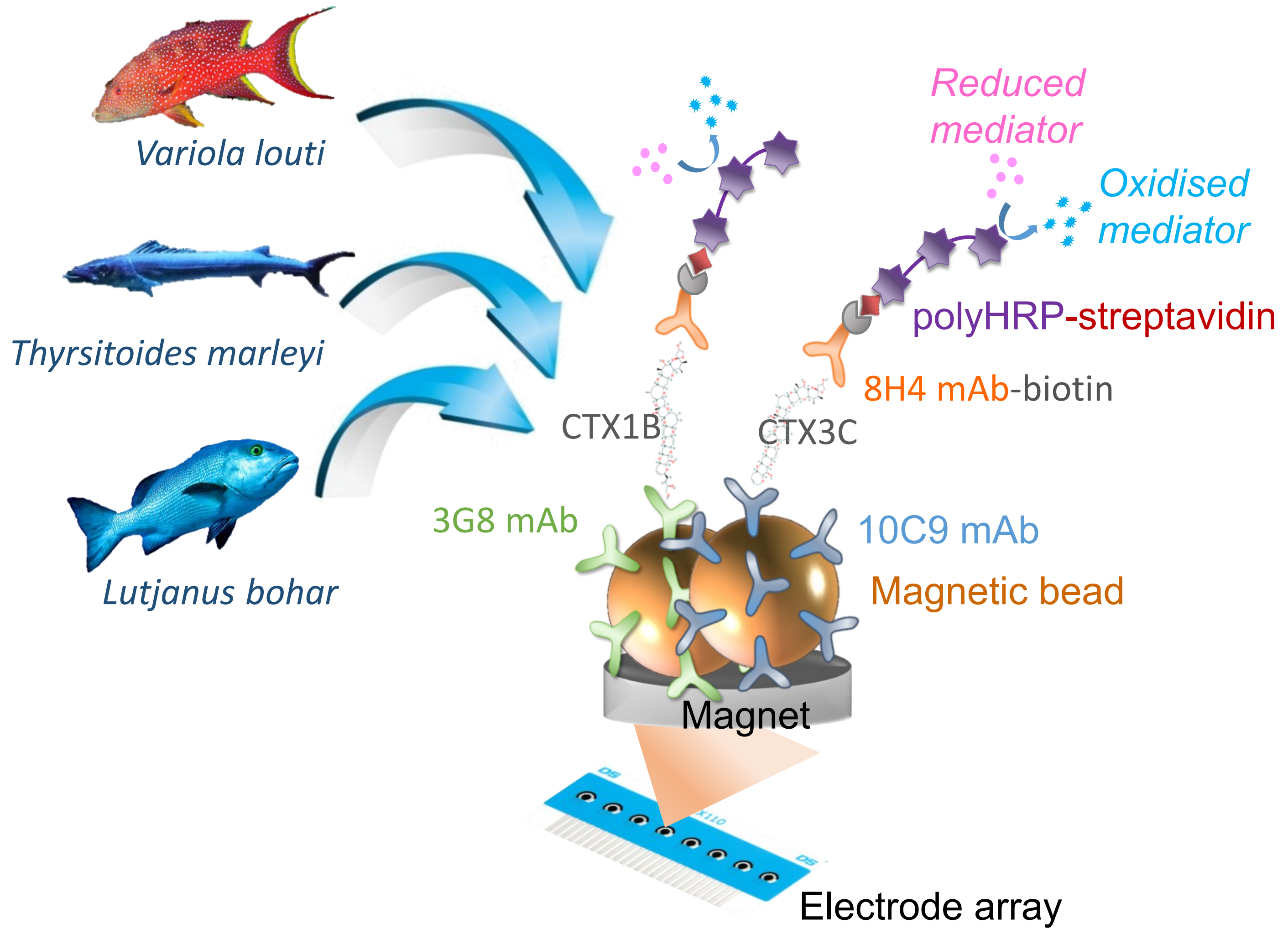


Figure 4: Extracted ion chromatogram of C-CTX-1 standard (top) and a representative fish extract of *Lutjanus* sp. from event 10 (bottom), showing two precoursor/product confirmatory ion transitions.

CTX detection with antibodies:

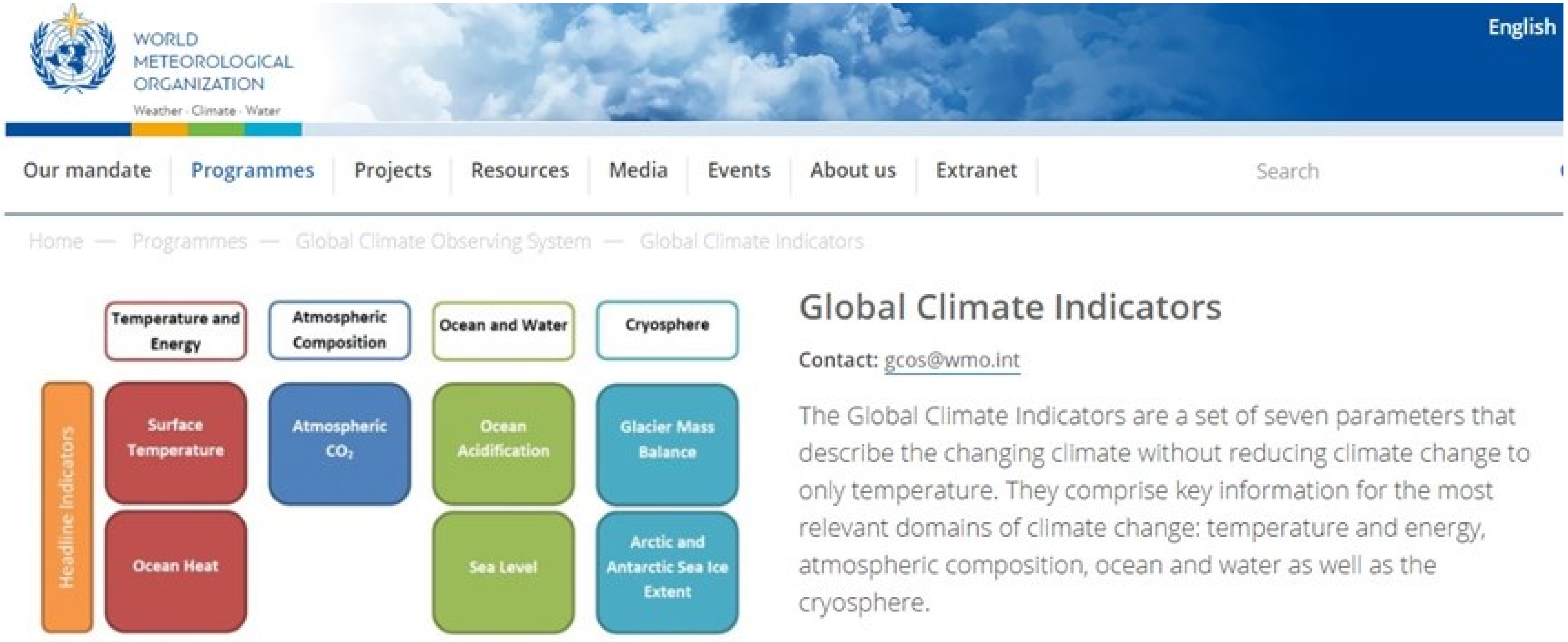


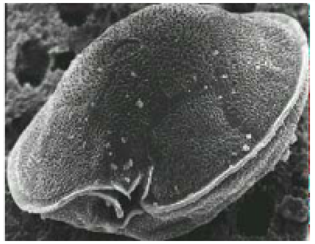
HABs and Climate Change

- Uncertainty on the potential impact CC may present on HABs.

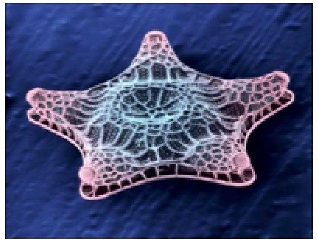
- Several communities / species of phytoplankton and benthic microalgae (HAB related and not HAB related) can be affected by factors affected by CC: Temperature, pH, sea rise, precipitation and freshwater runoff (including nutrient balance) for example.

- Additional influence of Global Change

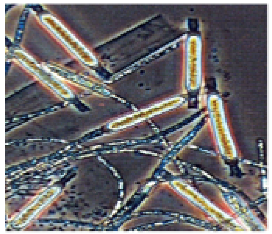




dinoflagellates



diatoms

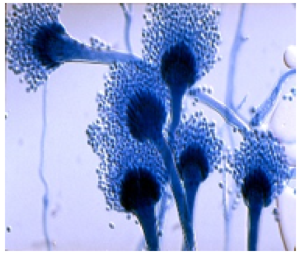


cyanobacteria

parasites

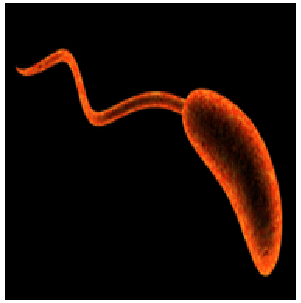
Occurrence, intensity, species composition, toxicity

Invasive species

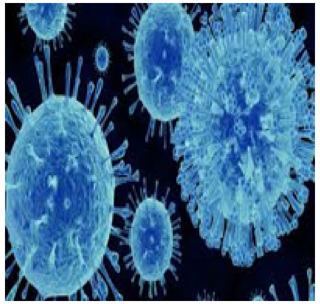


fungi

vectors



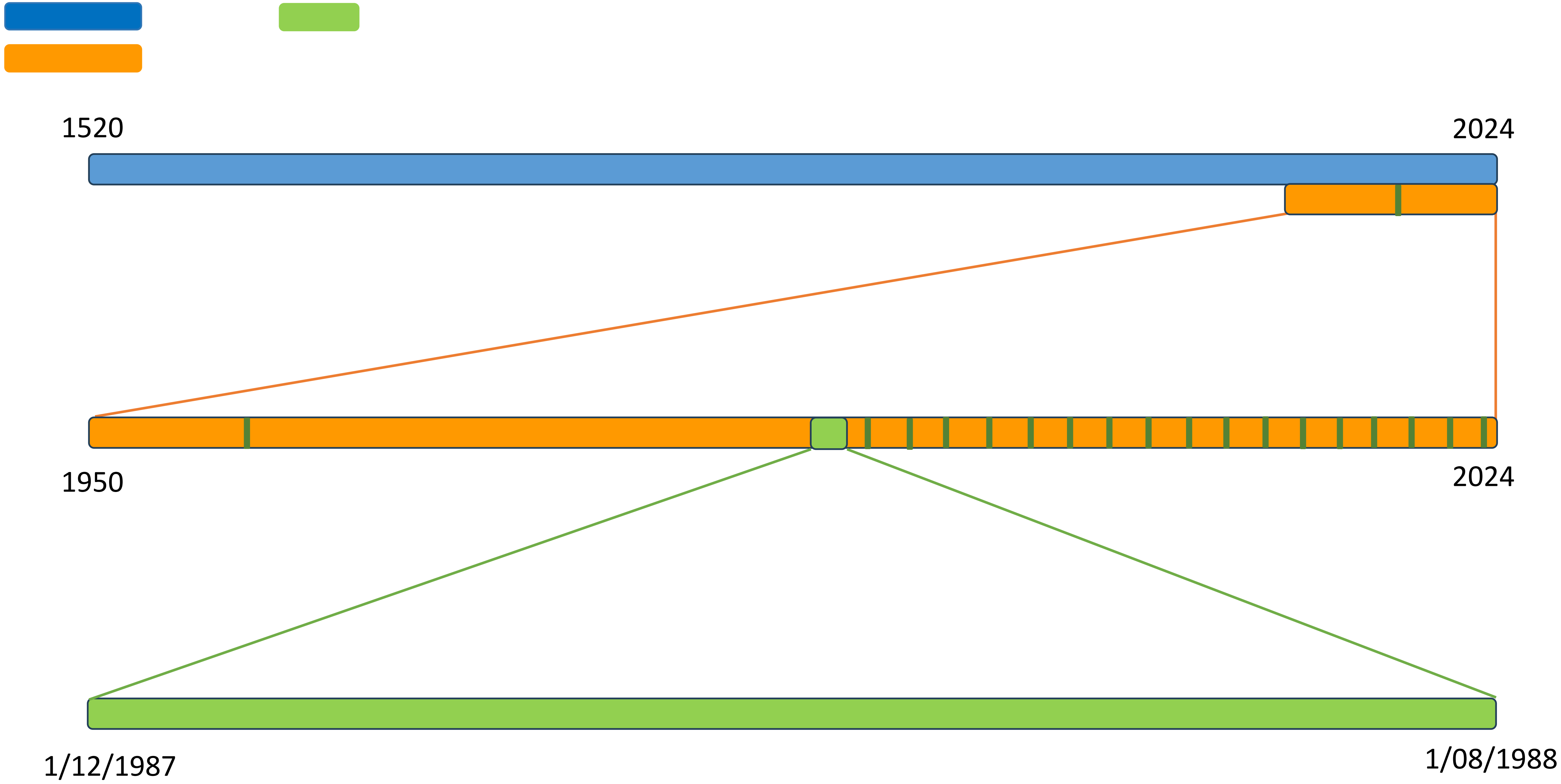
bacteria



viruses

protozoa

Ciguatera versus ASP:

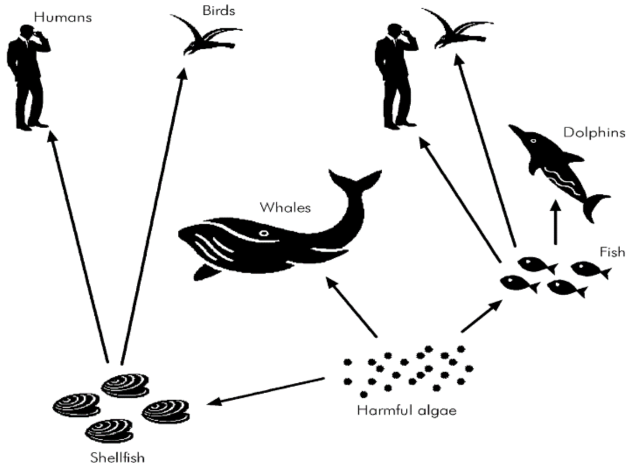


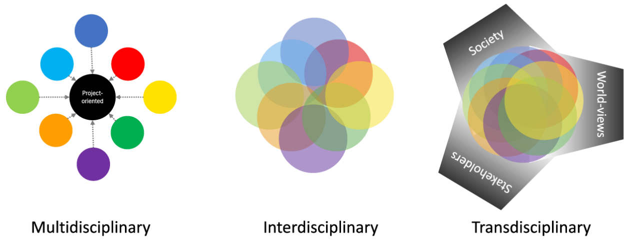
ASP and CIGUATERA AWARENESS:

IMPACT	CIGUATERA		ASP	
Awareness	1520	🟠	1987	🟢
Research	1950's	🟠	1958/1987	🟢
Definition of case	SEVERAL	🟠	YES	🟢
Present epidemiological records	HIGH	🔴	SCARCE	🟢
Effective dose	UNCERTAIN	🟠	CONSENSUS	🟢
Chronic effects	YES	🟠	YES	🟠
Effect on fauna	LOW?	🟠	HIGH	🟠
Massive environmental impact	DUBIOUS	🟠	HIGH	🟠
Climate change implications	YES	🟠	YES	🟠
TOXINS				
Causative toxins	SOME	🟠	YES	🟢
Toxin structures	SOME	🟠	YES	🟢
Complexity of toxin family	HIGH	🟠	LOW	🟢
Toxin mechanism of action	SOME	🟠	YES	🟠
Toxicity equivalent factors among toxin analogues	SOME	🟠	SOME	🟢
TOXIN PRODUCERS AND WEBS				
Toxin production organisms	COMPLEX	🟠	SIMPLE	🟢
Ecology of toxin producer organisms	COMPLEX	🟠	COMPLEX	🟠
Toxin transfer within the food webs	COMPLEX	🟠	SIMPLE	🟠
Variety of toxic seafood	HIGH	🟠	LOW	🟢

TOXIN ANALYSIS	CIGUATERA		ASP	
Availability of standards	SOME	🟠	YES	🟢
Availability of certified standards	NO	🔴	YES	🟢
Availability of reference material	SCARCE	🔴	YES	🟢
Validated toxin extraction method	NO	🟠	YES	🟢
Consensus on analytical method	SOME	🟠	YES	🟢
Validated analytical method	NO	🟠	YES	🟢
MANAGEMENT				
Regulation	SCARCE	🔴	YES	🟢
Maximum permitted level	NO	🔴	YES	🟠
Guidance levels	SOME	🟠	YES	🟠
Official method	NO	🟠	YES	🟢
Monitoring programmes for toxin producing organisms	SCARCE	🟠	YES	🟠
Monitoring programmes for toxins in seafood	SCARCE	🟠	YES	🟢

Thank you !





IMPACT	**CIGUATERA**		**ASP**	
Awareness	1520	orange	1987	green
Research	1950's	orange	1958/1987	green
Definition of case	SEVERAL	orange	YES	green
Present epidemiological records	HIGH	red	SCARCE	green
Effective dose	UNCERTAIN	orange	CONSENSUS	green
Chronic effects	YES	orange	YES	orange
Effect on fauna	LOW?	orange	HIGH	orange
Massive environmental impact	DUBIOUS	orange	HIGH	orange
Climate change implications	YES	orange	YES	orange
TOXINS				
Causative toxins	SOME	orange	YES	green
Toxin structures	SOME	orange	YES	green
Complexity of toxin family	HIGH	orange	LOW	green
Toxin mechanism of action	SOME	orange	YES	orange
Toxicity equivalent factors among toxin analogues	SOME	orange	SOME	green
TOXIN PRODUCERS AND WEBS				
Toxin production organisms	COMPLEX	orange	SIMPLE	green
Ecology of toxin producer organisms	COMPLEX	orange	COMPLEX	orange
Toxin transfer within the food webs	COMPLEX	orange	SIMPLE	orange
Variety of toxic seafood	HIGH	orange	LOW	green

TOXIN ANALYSIS	**CIGUATERA**		**ASP**	
Availability of standards	SOME	orange	YES	green
Availability of certified standards	NO	red	YES	green
Availability of reference material	SCARCE	red	YES	green
Validated toxin extraction method	NO	orange	YES	green
Consensus on analytical method	SOME	orange	YES	green
Validated analytical method	NO	orange	YES	green
MANAGEMENT				
Regulation	SCARCE	red	YES	green
Maximum permitted level	NO	red	YES	orange
Guidance levels	SOME	orange	YES	orange
Official method	NO	orange	YES	green
Monitoring programmes for toxin producing organisms	SCARCE	orange	YES	orange
Monitoring programmes for toxins in seafood	SCARCE	orange	YES	green

Marine toxin recognition strategies
Preventive measures.

Identify risks ahead: toxin producing microorganisms, involved toxins, toxins in fish, fish distribution, sentinel species, reference stations, temporal distribution

Clinical and epidemiology: case descriptions, awareness by the medical community, report, gather data, gather and preserve food samples and patients biological samples.

Analysis and research: Well-equipped laboratories, skilled analysts, set-up analytical methods for different matrixes, consider international collaborations ahead.

Otherwise… run and hide!