Biotoxin hazards associated with bivalve mollusc consumption

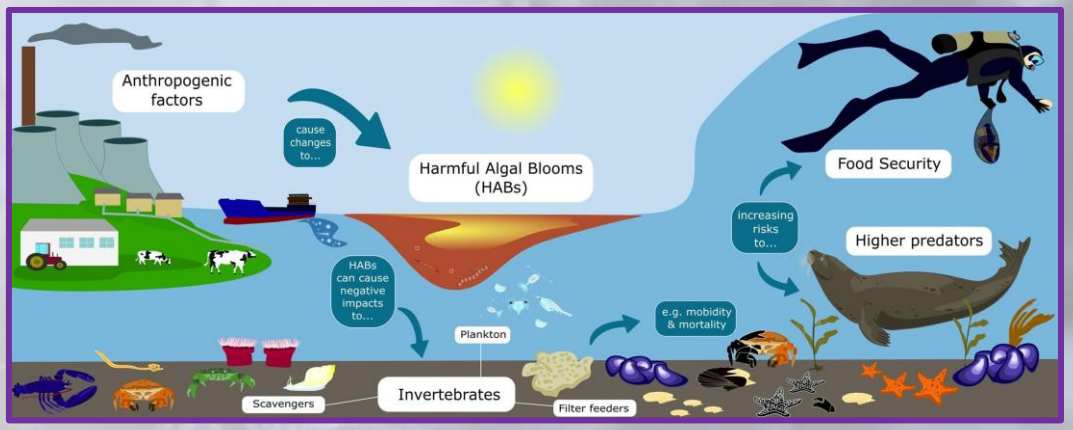
Dr Andrew Turner

Cefas

Biotoxin hazards associated with bivalve mollusc consumption

- **Overview**

- Introduction to Harmful Algal Blooms
- Regulated marine toxins
- Health impacts
- Changes and emerging hazards

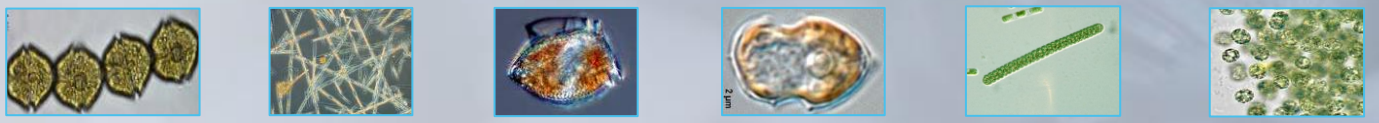


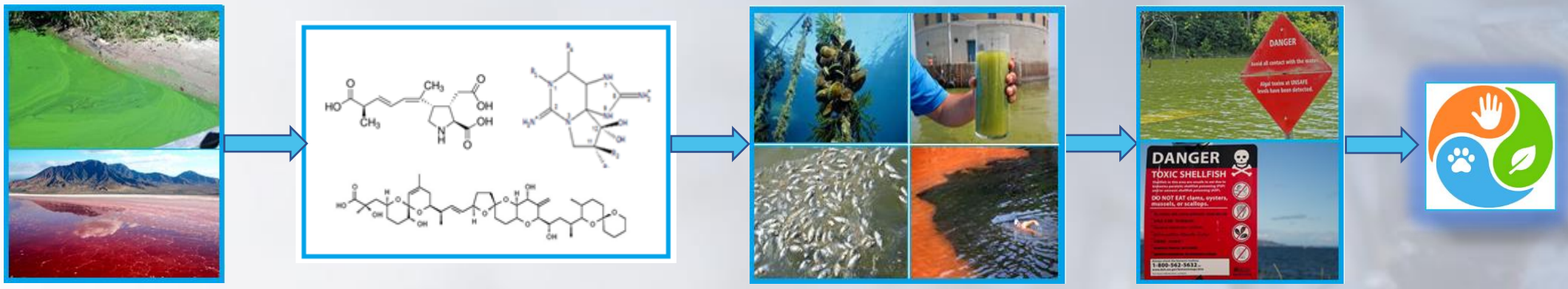
Cefas Natural Aquatic Toxins Team

Cefas
INTERNATIONAL CENTRES OF EXCELLENCE
SEAFOOD SAFETY

- Official control testing of molluscs in Great Britain
- Research activities in toxins field
- Collaborative science with other countries
- Active publishers of new toxin science
- Reference materials, ring trials
- Method validation activities
- Marine and freshwater
- Invertebrates, fish, water and algae/bacteria
- “One Health” approach

Algal blooms





One Health impacts

Human health

- Shellfish toxin poisonings
- Ciguatera Fish Poisoning (CFP)
- Toxin aerosols – respiratory illness
- Recreational exposure sickness
- Drinking water poisoning

~60,000 annual intoxications (~1.5% mortality)*

Heat-stable; no antidotes; no clinical tests; sub-ppb levels can cause toxicity

Animal Health

- Marine mammal deaths
- Aquatic bird deaths
- Fish kills
- Invertebrate health/growth/reproduction
- Drinking water poisoning
- Genetic impacts

Increasing global reports of morbidities & mortalities

Ecosystem Health

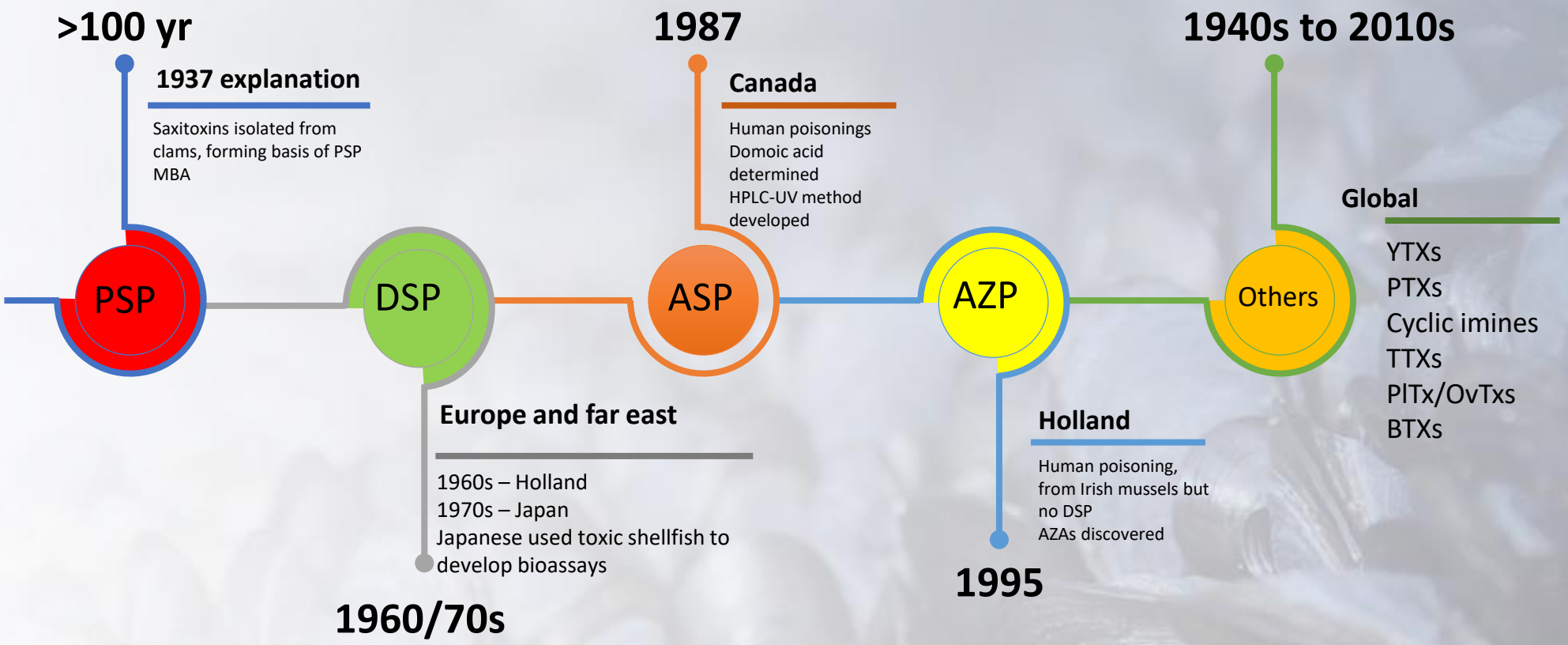
- Mass mortalities
- Aquatic chemistry changes
- Population structures
- Behavioural changes
- Habitat/substrate changes
- Ecosystem recovery rates

1/8 of “marine disturbances” from toxin exposure**

*Kantiani, L., *et al.* 2010. Emerging food contaminants: A review. *Anal. Bioanal. Chem.* https://doi.org/10.1007/s00216-010- 3944-9.

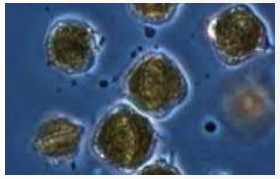
**Sherman, B.H., 2000. Marine ecosystem health as an expression of morbidity, mortality and disease events. *Mar. Pollut. Bull*. https://doi.org/10.1016/S0025-326X(00) 00113-2.

A brief history of shellfish toxin discovery

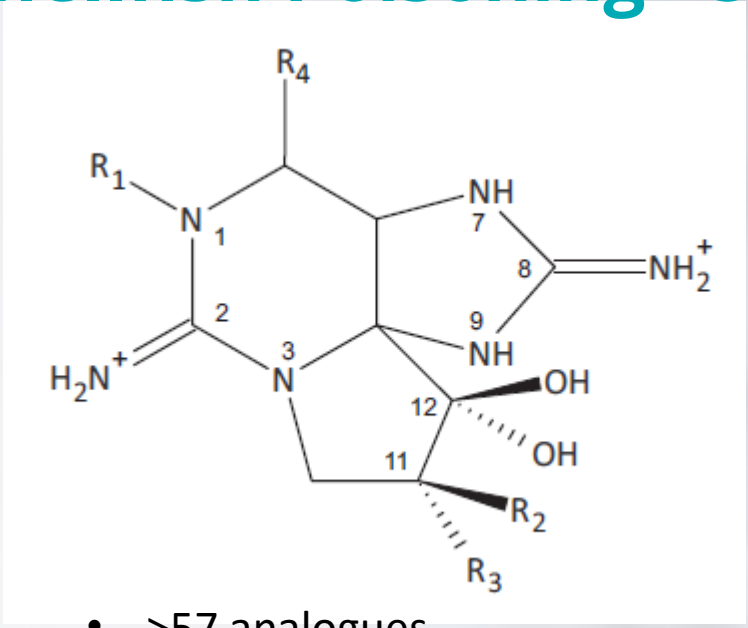


1947 – Brevetoxins (Gulf of Mexico); 1986 – Yessotoxins (Japan); 1989 – Pectenotoxins (Japan); 1980s – Palytoxin/ovatoxins (Hawaii, Japan, Mediterranean); 1990s – Cyclic imines (Canada, NZ); 2000s – Tetrodotoxins (Japan, NZ, UK);

Paralytic Shellfish Poisoning - Saxitoxins



- *Alexandrium* sp.
- *Gymnodinium catenatum*
- *Pyrodinium bahamense*



- >57 analogues
- Wide ranging toxicities
- Mostly hydrophilic
- Variable charge states

- Blocks voltage-dependent Na channels in motor nerves
- Tingling/numbness ⟶ muscle paralysis and death

Human health

- Primary cause of marine toxin related mortality
- ~2,000 intoxications pa; ~15% mortality
- Global distribution (Arctic to temperate southern hemisphere)
- 800 µg STX eq/kg MPL
- UK >27,000 µg STX eq/kg ; Global > 1 million

Animal Health

- Mass mortalities of some bivalve species
- Seabird deaths
- Marine mammal deaths
- Sub-lethal & genetic chronic effects

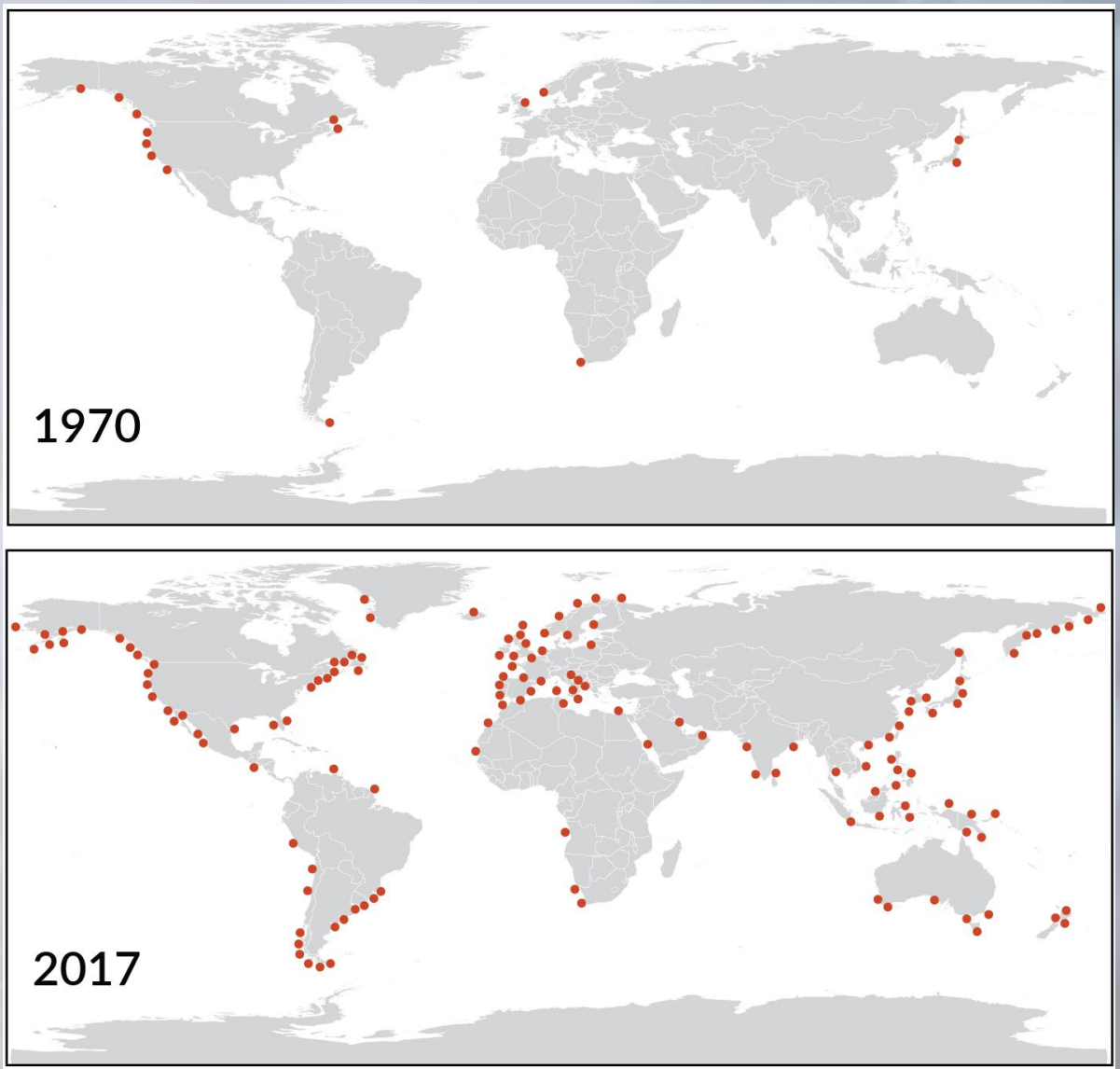
Ecosystem Health

- Trophic impacts following die-offs
- Entire trophic web can be affected (from plankton to whales)

Global distribution of PSP

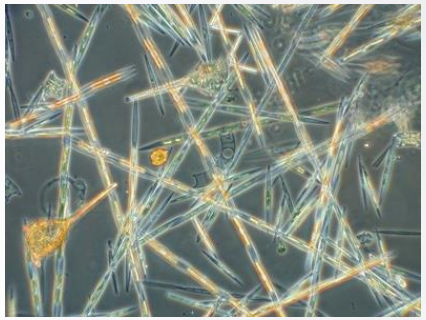
- Notable changes over time

- Increased/improved monitoring and detection capabilities
- AND/OR
- Global expansion of HABs
  - Anthropogenic inputs
  - Environmental/climatic change
  - Water/shellfish stock movements

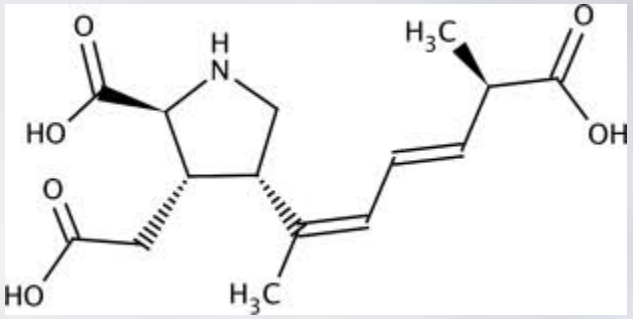


Courtesy of U.S. National Office for Harmful Algal Blooms (https://hab.whoi.edu/maps/regions-world-distribution)

Amnesic Shellfish Poisoning – Domoic acid(s)



- *Pseudo-nitzchia* sp.



- Neuroexcitatory amino acid
- Nine isomers

- Antagonistic effects at glutamate receptor, neuron damage activation of AMPA & kainate receptors
- Gastric, neurological, cardiac and memory loss + death

Human health

- 1987 Canada outbreak, including fatalities
- No subsequent known poisonings
- Causative diatoms found globally
- 20 mg/kg MPL
- UK >50 mg/kg

Animal Health

- 1961 California – "chaotic, attacking seabirds"
- Behavioural changes in sea mammals
- Widespread seabird/mammal mortalities
- Sub-lethal chronic exposure effects

Ecosystem Health

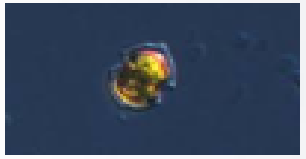
- Mass mortality impacts on trophic web

ASP global distribution

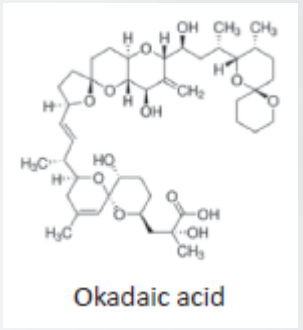
- 2016 map
- Temperate oceanic zones
- Aus/NZ
- Sampling bias likely
- Apparent near-absence in Latin America

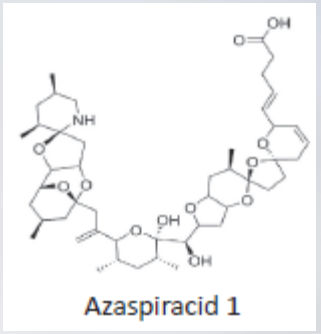
Courtesy of U.S. National Office for Harmful Algal Blooms (https://hab.whoi.edu/maps/regions-world-distribution)

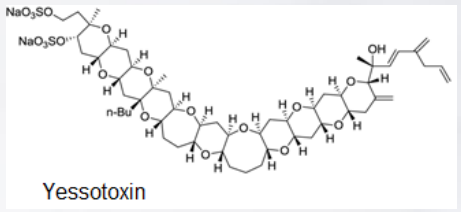
Diarrhetic Shellfish Poisoning – Lipophilic Toxins

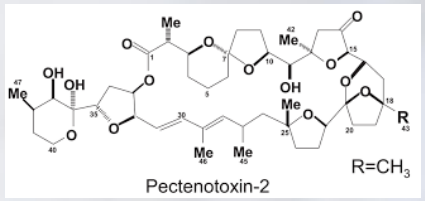


- *Dinophysis* sp.
- *Prorocentrum* sp.
- *Azadinium* sp.
- *Amphidoma* sp.
- *Lingolodinium* sp.



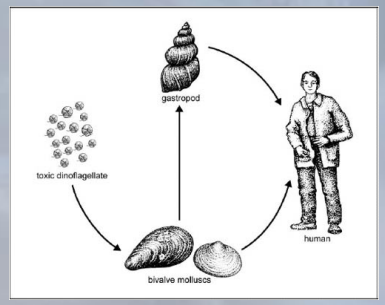






- 11 OA analogues
- >60 AZAs
- Multiple YTXs/PTXs

- Inhibition of protein phosphatases
- Intense gastric symptoms (temporary), tumour promotion



Human health

- DSP = Most common shellfish toxin poisoning
- 2001-2015 ~1200 reported intoxications
- 3 outbreaks in UK in recent years
- Actual impact much higher, lack of clear symptoms
- AZP – most recent ~200 intoxications to date
- YTXs/PTXs – no known acute health impacts

Animal Health

- Yessotoxin impact on mass invertebrate mortalities

Ecosystem Health

- Trophic impacts following die-offs

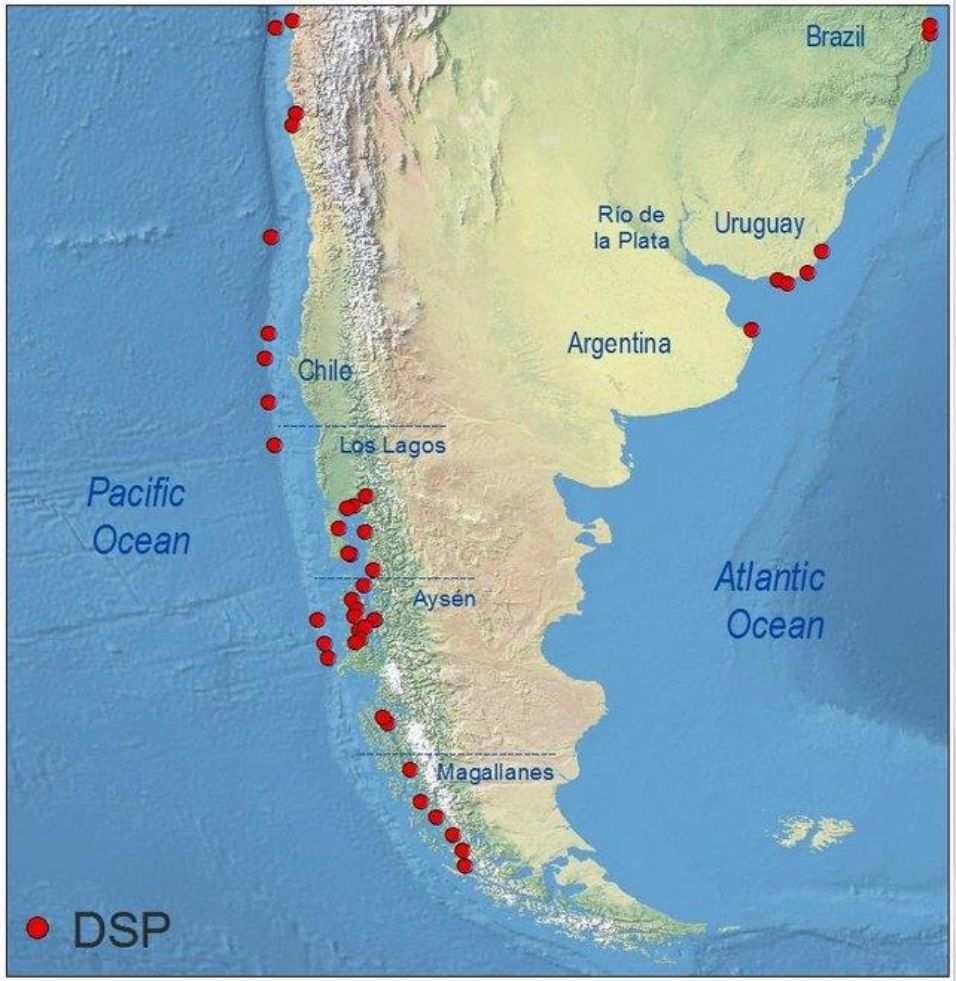
Global distribution of DSP

Global distribution Dinophysis toxin detection (ICES-IOC HAEDAT, 2014)

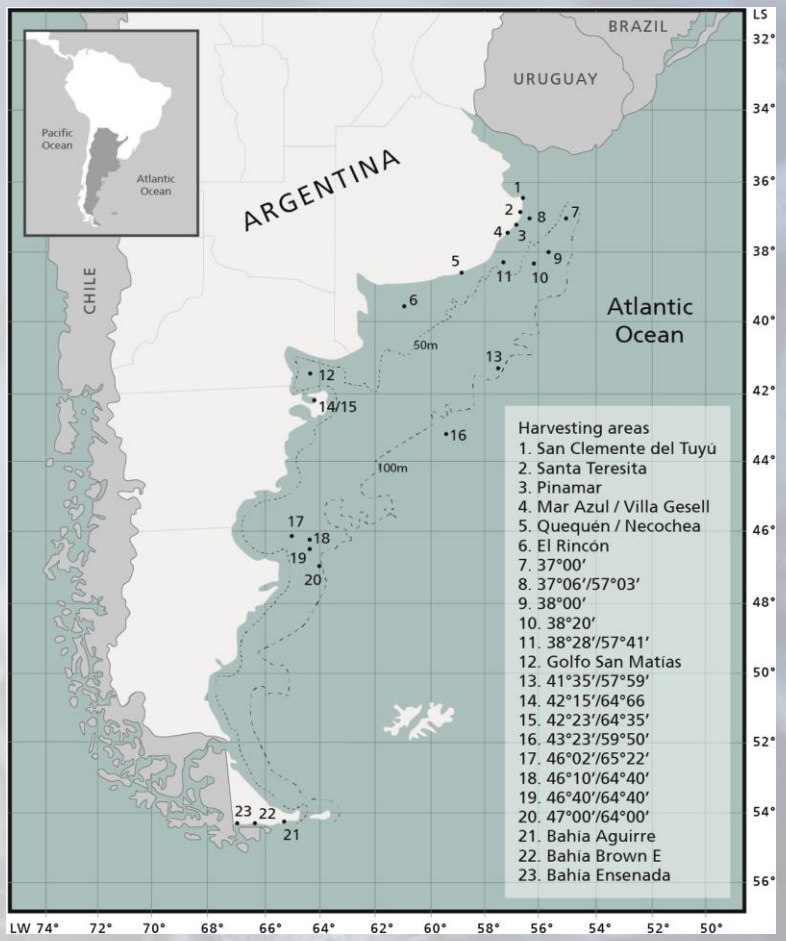
Courtesy of U.S. National Office for Harmful Algal Blooms
(https://hab.whoi.edu/maps/regions-world-distribution)

Latin America - DSP distribution

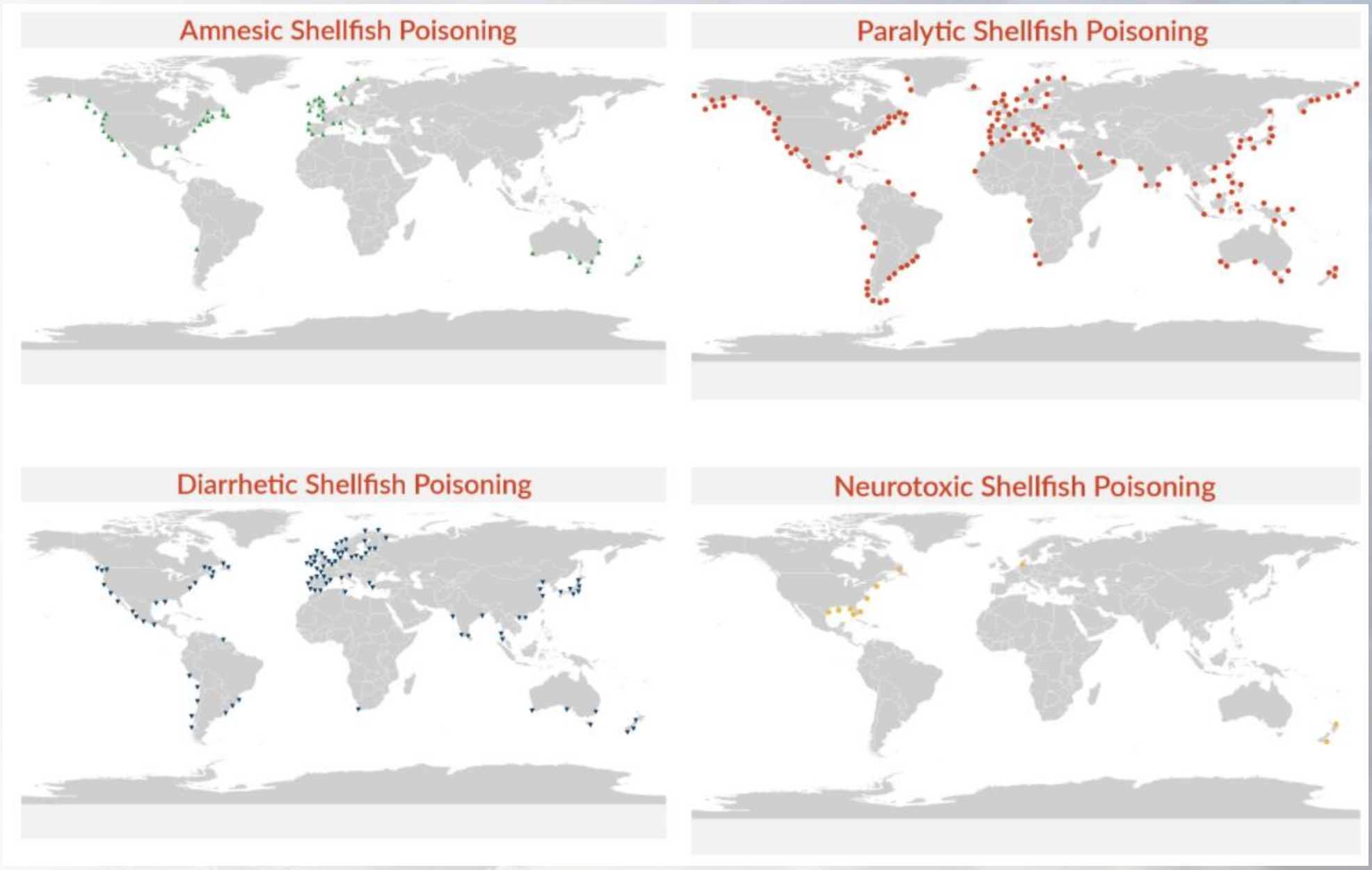
2014 - HAEDAT



2015 – Argentina (Turner and



Overall (2016)



UK Official control results

Test (2001 -)	Number	Total
PSP MBA	7,354	46,455 PSP
PSP HPLC Screen	37,804	
DSP MBA	22,549	51,348 DSP
LT LCMSMS	28,799	
ASP	23,653	23,653 ASP
Phyto (2005-)	13,836 (E&W)	

Test	> MPL
PSP	255 (0.6%)
DSP	2,775* (5.4%)
ASP	28 (0.1%)
AZA	47 (0.2%)

*includes atypical DSP MBA E&W

Known intoxications

- ASP – none known
- PSP – 1968 (78 hospitalised)
- DSP – four known events (1997-2019)
- AZP – none known

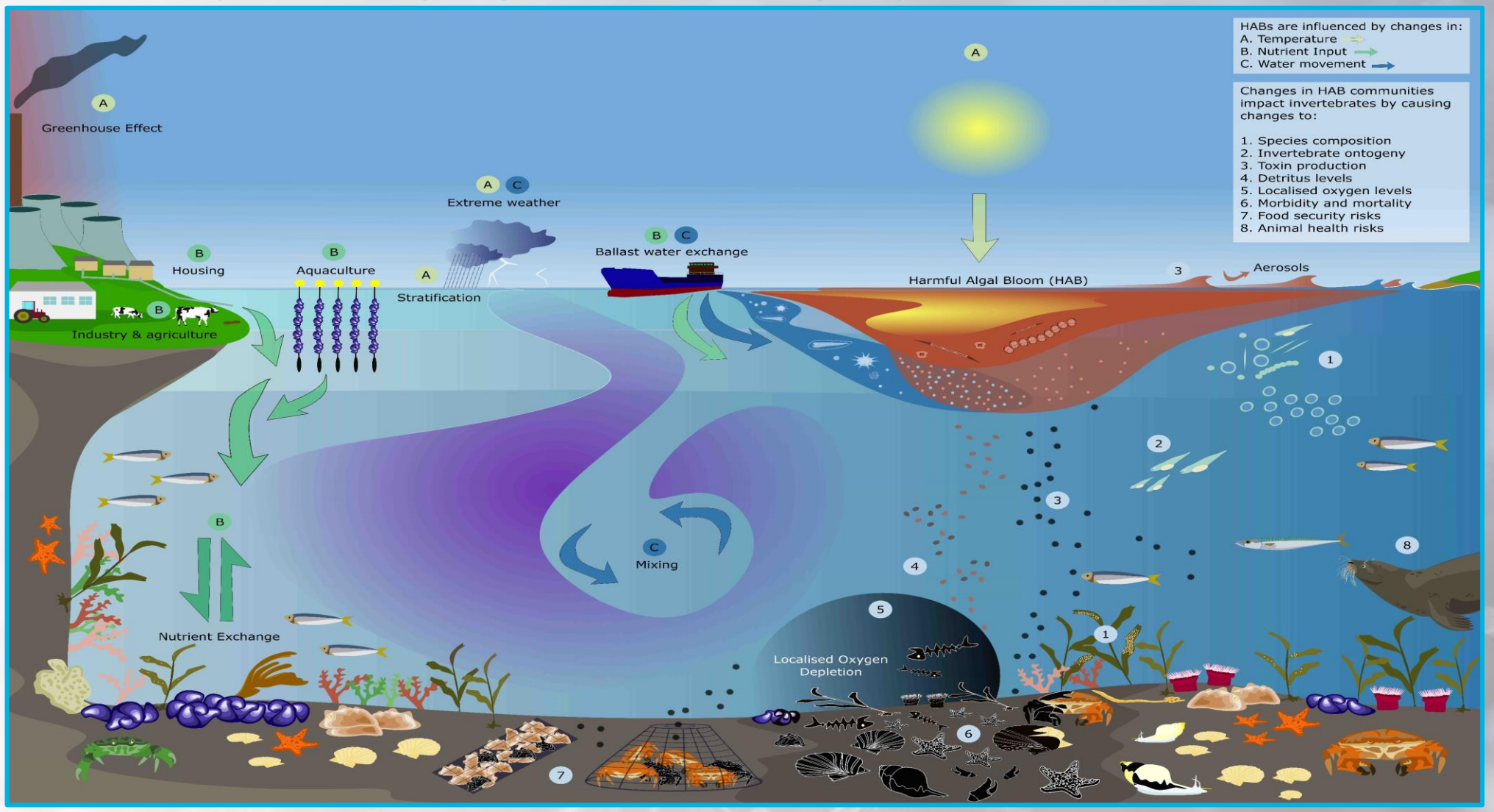
DSP UK intoxications from mussels –

Poisonings occasionally happen even with official monitoring established

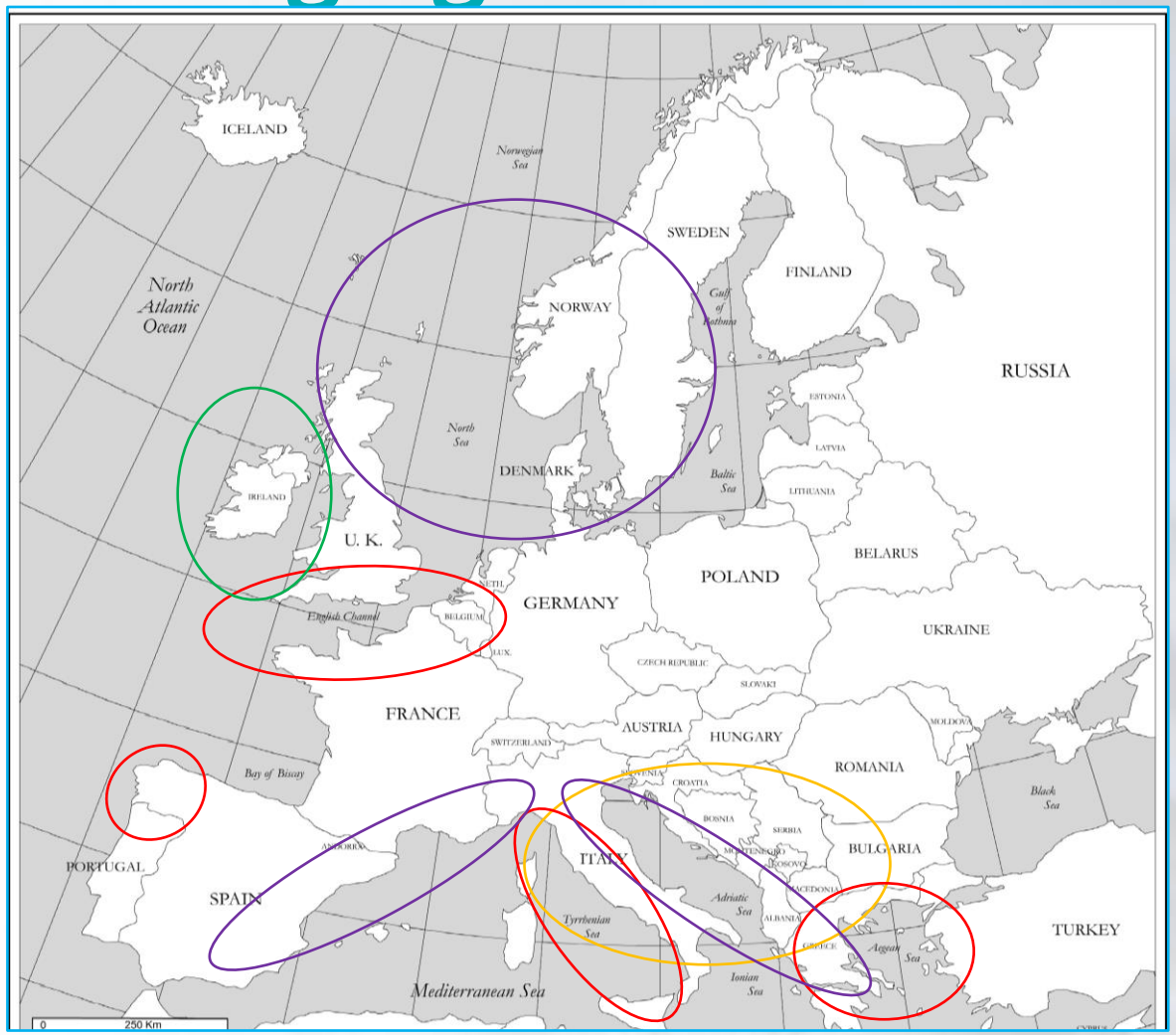
Date	Source	Affected	Reasons	EPT?
1997	UK	49 people	Unknown	Not available
June 2006	Scotland	171 people	Missed sampling	None
July 2013	Shetland	~70 people	Sudden increase in toxicity between OC tests	None
June 2019	Cornwall	3 confirmed, 3 probable	Sudden increase in toxicity between OC tests	None

- In all cases, end product testing use would prevent consumption of contaminated products

Changes bringing new/emerging hazards and risks



Emerging/unknown toxin threats



- Tetrodotoxins
- AZA analogues
- Cyclic imines
- Palytoxins
- Brevetoxins

Target analysis using LC–MS/MS

RAPID COMMUNICATIONS

Detection of the pufferfish toxin tetrodotoxin in European bivalves, England, 2013 to 2014

A D Turner (andrew.turner@cefas.co.uk)[1], A Powell[1], A Schofield[1,2], D N Lees[1], C Baker-Austin[1]
1. Food Safety Group, Centre for Environment Fisheries and Aquaculture Science, Weymouth, Dorset, United Kingdom
2. Department of Chemistry, University of Hull, Hull, United Kingdom

Mar. Drugs **2015**, *13*, 1224-1254; doi:10.3390/md13031224
marine drugs
ISSN 1660-3397
www.mdpi.com/journal/marinedrugs

Review

Potential Threats Posed by New or Emerging Marine Biotoxins in UK Waters and Examination of Detection Methodology Used in Their Control: Brevetoxins

Andrew D. Turner [1,*], Cowan Higgins [2], Keith Davidson [3], Andrea Veszelovszki [3], Daniel Payne [1,4], James Hungerford [5] and Wendy Higman [1]

Chemosphere 215 (2019) 881–892
Contents lists available at ScienceDirect
Chemosphere
ELSEVIER
journal homepage: www.elsevier.com/locate/chemosphere

First detection of tetrodotoxin and high levels of paralytic shellfish poisoning toxins in shellfish from Sicily (Italy) by three different analytical methods

Carmela Dell'Aversano [a, b, *], Luciana Tartaglione [a, b], Giuseppe Polito [a], Karl Dean [c], Mariagrazia Giacobbe [d], Silvia Casabianca [b, e], Samuela Capellacci [b, e], Antonella Penna [b, e], Andrew D. Turner [c]

marine drugs
MDPI

Article

Detection of Tetrodotoxin Shellfish Poisoning (TSP) Toxins and Causative Factors in Bivalve Molluscs from the UK

Andrew D. Turner [1,*], Monika Dhanji-Rapkova [1], Lewis Coates [1], Lesley Bickerstaff [1], Steve Milligan [1], Alison O'Neill [1], Dermot Faulkner [2], Hugh McEneny [2], Craig Baker-Austin [1], David N. Lees [1] and Myriam Algoet [1]

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Review

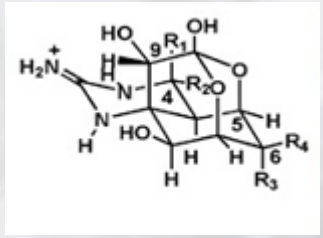
Potential Threats Posed by New or Emerging Marine Biotoxins in UK Waters and Examination of Detection Methodologies Used for Their Control: Cyclic Imines

Keith Davidson [1,*], Clothilde Baker [2], Cowan Higgins [3], Wendy Higman [2], Sarah Swan [1], Andrea Veszelovszki [1] and Andrew D. Turner [2]

Example 1 – Pufferfish poisoning toxins in European molluscs

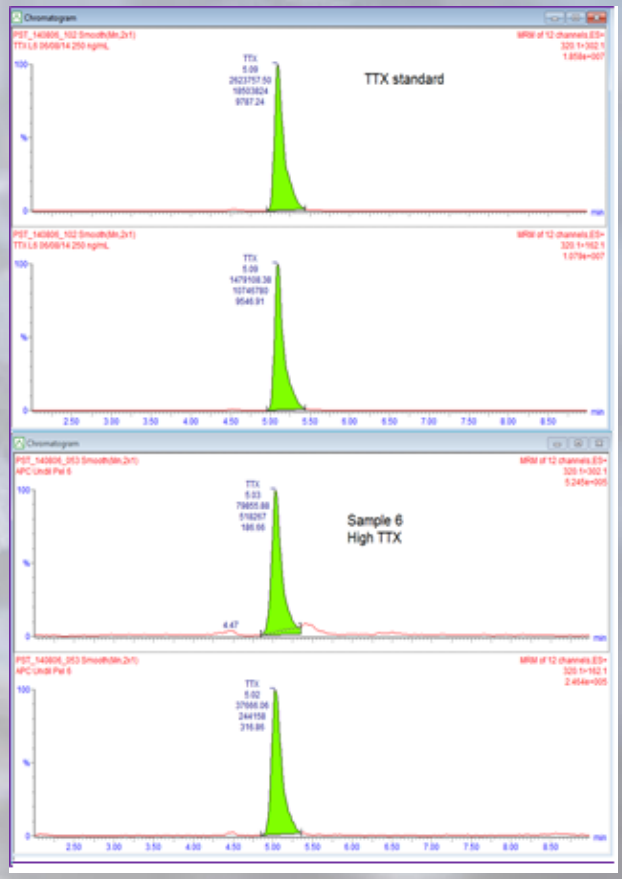
Fish, Crustaceans, Amphibians, Octopus, Sea slugs, Gastropods, Newts

- Tetrodotoxins (TTXs)
- Na Ch receptors
- Most marine toxins are algal
- TTXs thoughts to originate from bacteria e.g. *Vibrio* sp.
- Biosynthetic pathway unknown
- *Vibrio parahaemolyticus* in UK
- Possible TTX in molluscs???



Southern England

- *Vibrio* sp. in shellfish
- TTXs in shellfish flesh
- *Vibrio* isolated and cultured
- TTXs in cultures
- First evidence for TTXs in European bivalves
- Further evidence for TTX association with bacteria



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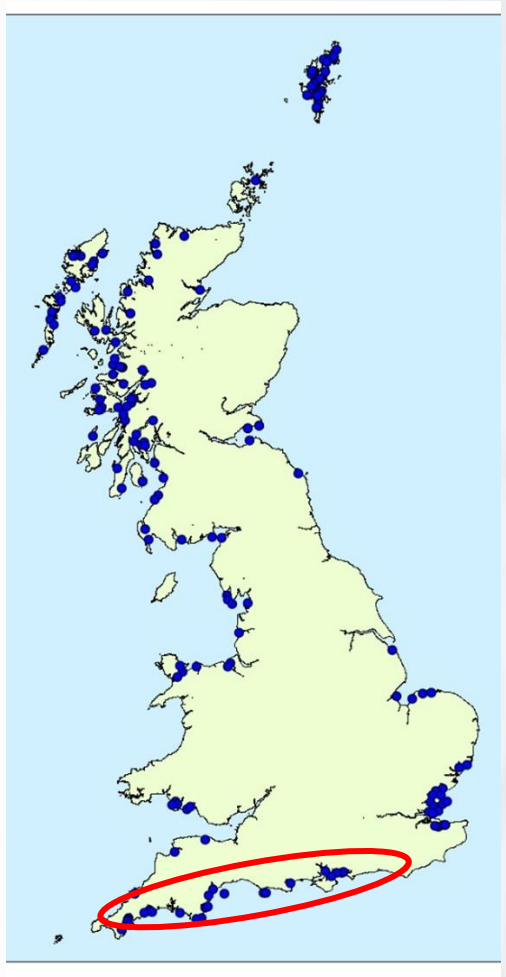
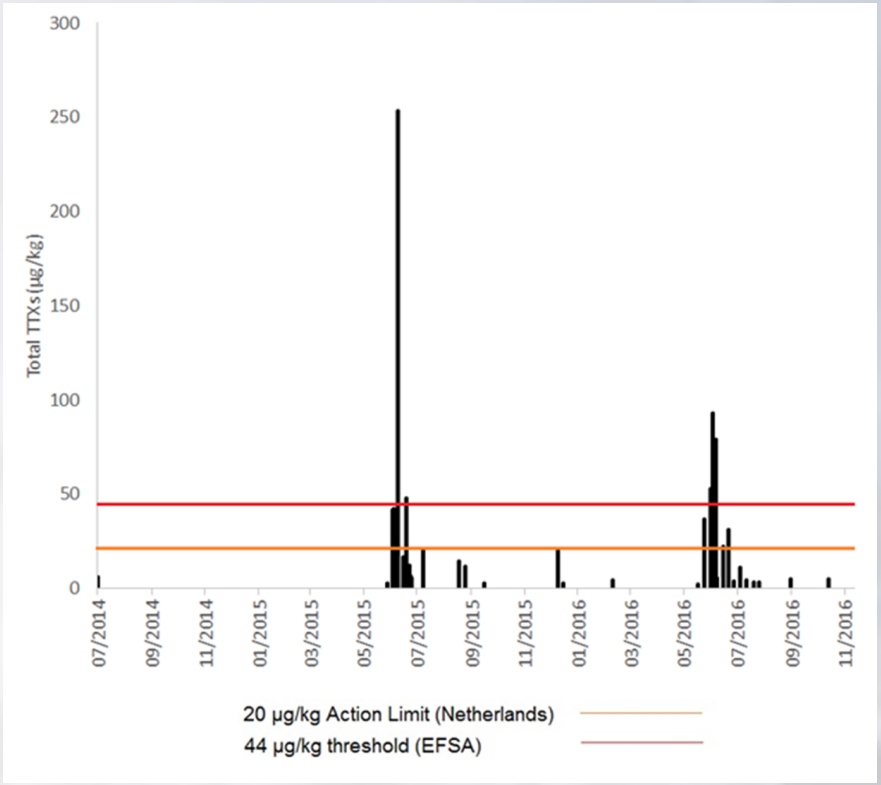
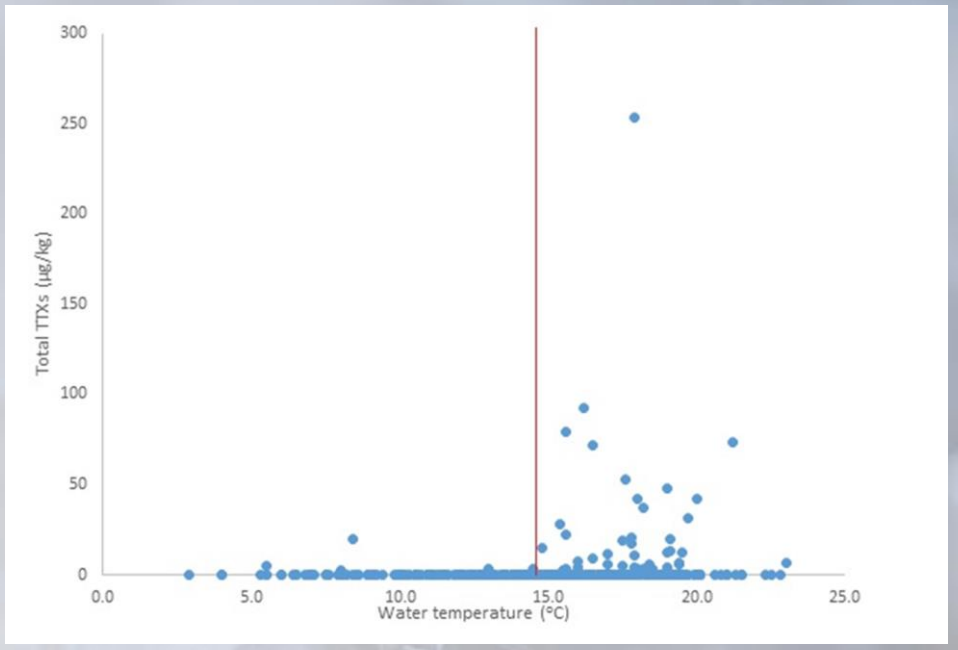




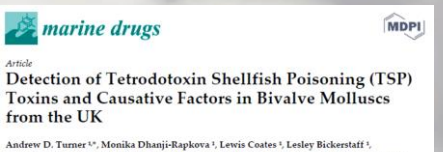
- Southern England
- Inshore waters
- Shallow, estuarine
- 15 °C threshold



- England, Netherlands, Greece
- N. Italy
- S. Italy, Galicia, France
- EFSA guidance = 44 µg/kg
- No regulations exist yet (more data)


RAPID COMMUNICATIONS

Detection of the pufferfish toxin tetrodotoxin in European bivalves, England, 2013 to 2014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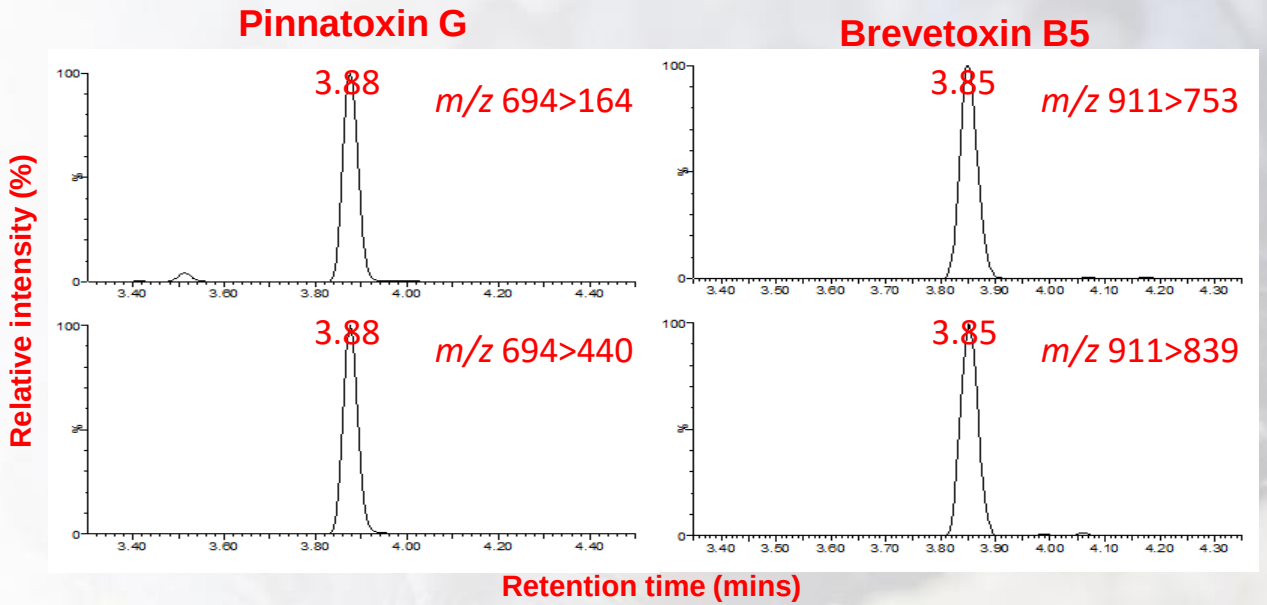
Pinnatoxins & Brevetoxins

- LC-MS/MS method for LTs extended
- Includes PnTx E, F, G
- Brevetoxins (BTX B2, B4, B5; PbTx2, PbTx3, S desoxy BTX B2,)
- Evidence for PnTx G noted in N. Europe



Brevetoxins from *Karenia brevis*

Until recently associated with USA and NZ event

Now found in parts of Mediterranean Sea

Pinnatoxins spread widely

Pinnatoxins & Brevetoxins

Brevetoxins from *Karenia brevis*

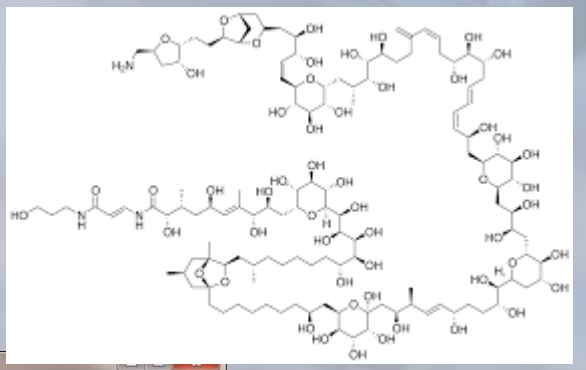
Until recently associated with USA and NZ event

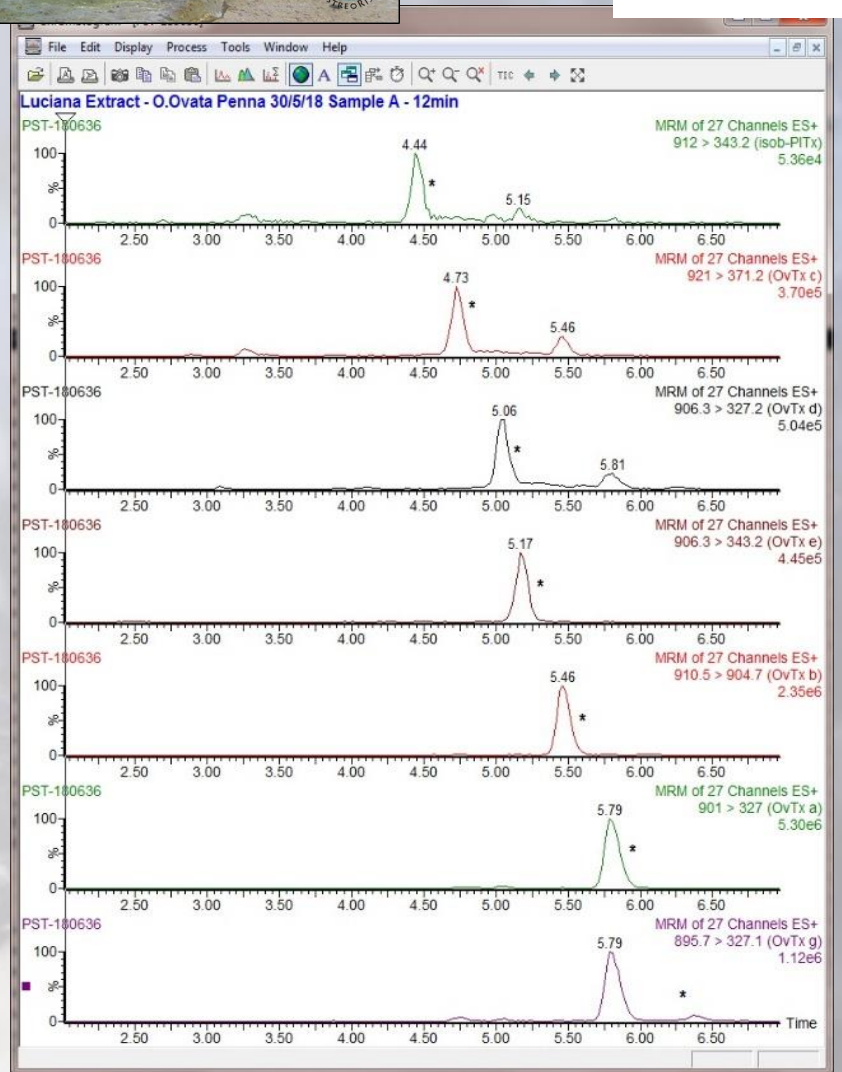
Now found in parts of Mediterranean Sea

Pinnatoxins spread widely

Palytoxins/Ovatoxins

- Issues in Mediterranean Sea + other regions
- LC with high resolution MS reported from Italy
- LC-MS/MS also useful
- Method developed and applied to European algal samples





Cyanotoxins

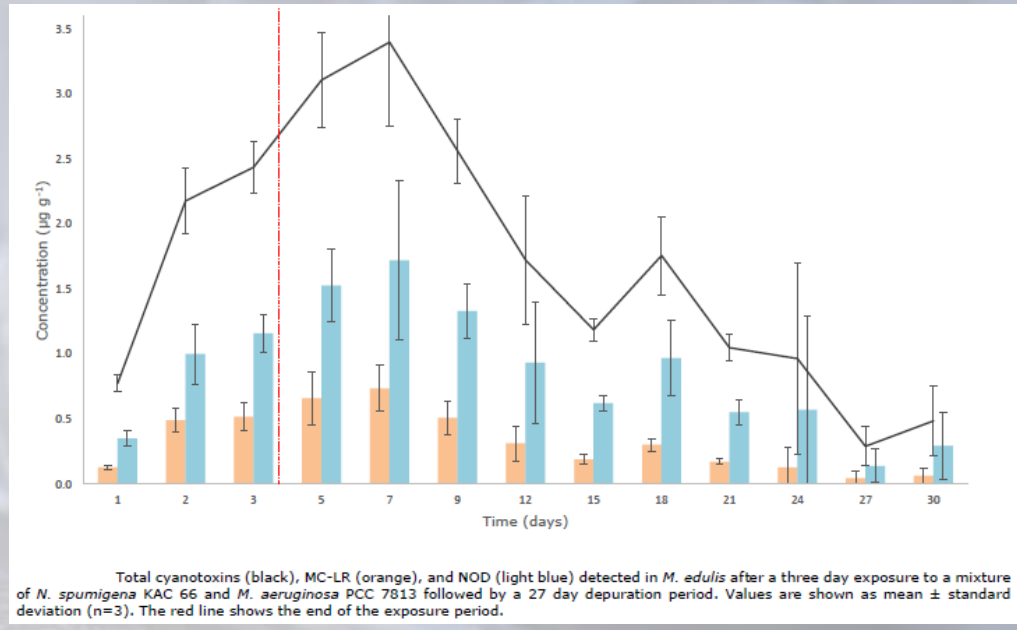
OPEN ACCESS Freely available online

PLoS one

Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters

Melissa A. Miller[1,2*], Raphael M. Kudela[2], Abdu Mekebri[3], Dave Crane[3], Stori C. Oates[1], M. Timothy Tinker[4], Michelle Staedler[5], Woutrina A. Miller[6], Sharon Toy-Choutka[1], Clare Dominik[7], Dane Hardin[7], Gregg Langlois[8], Michael Murray[5], Kim Ward[9], David A. Jessup[1]

- Wide range of toxin hazards from cyanobacteria
  - Microcystins
  - Nodularins
  - Cylindrospermopsins
  - Anatoxins
- Impacts on
  - Drinking water quality
  - Recreational activities
  - Food safety
- Otters
- Links between freshwater cyanobacterial blooms and estuarine shellfish beds
- Shellfish accumulation studies



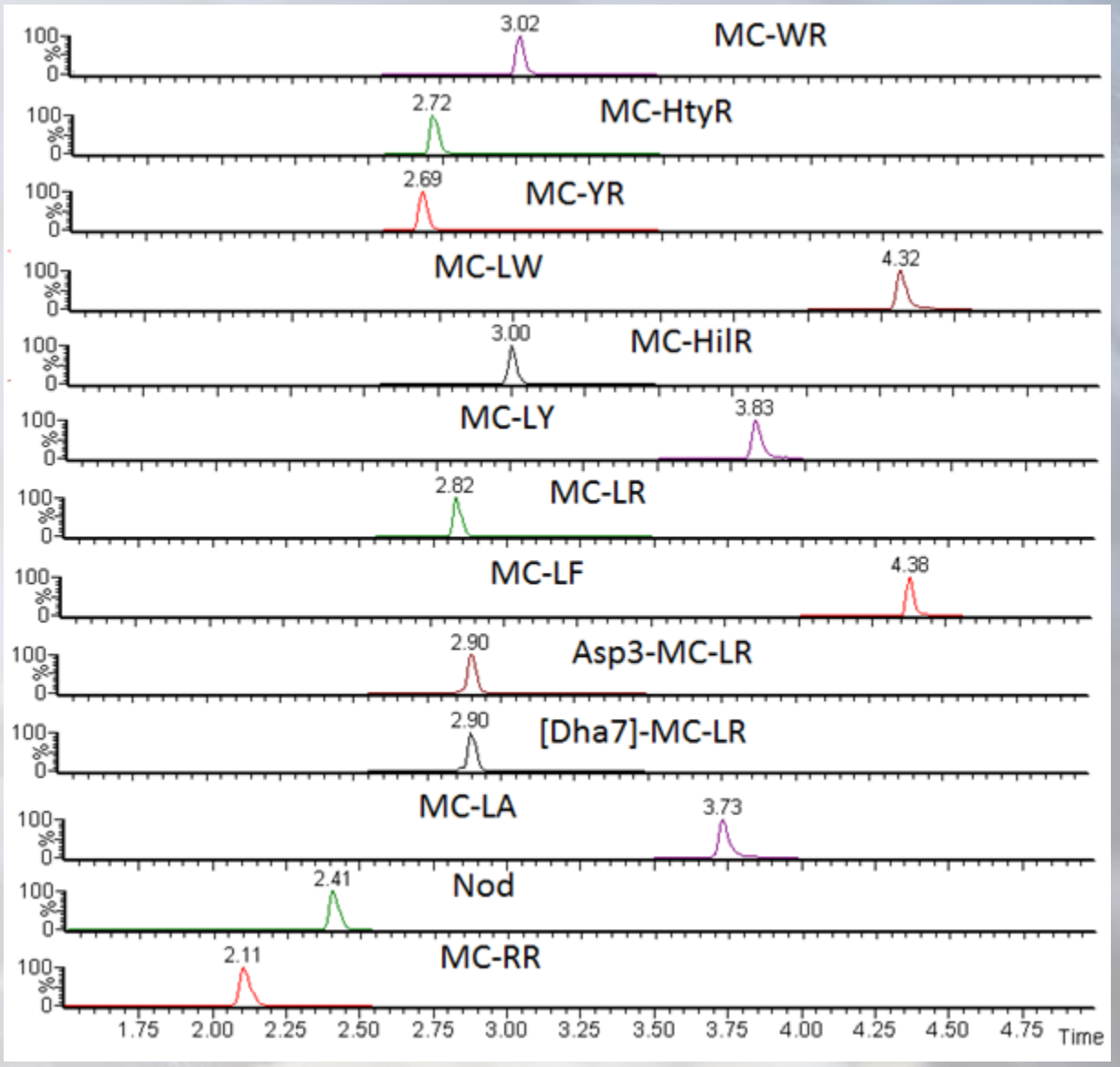
Total cyanotoxins (black), MC-LR (orange), and NOD (light blue) detected in *M. edulis* after a three day exposure to a mixture of *N. spumigena* KAC 66 and *M. aeruginosa* PCC 7813 followed by a 27 day depuration period. Values are shown as mean ± standard deviation (n=3). The red line shows the end of the exposure period.

Mussel exposure study – TDI exceeded by 500 times after 7 days accumulation (eating 20 mussels)
Slow depuration

Cyanotoxins

- LC-MS/MS
- 1.7 µm, 2.1x50 mm Waters Acquity UPLC BEH C18 column + guard
- Water
- Algae
- Shellfish
- Powders

- 5.5 min method



Overall

- Three main regulated groups – PSP, ASP, DSP (LTs)
- Brevetoxins regulated in some regions
- Emerging toxin threats not to be ignored
- Proper risk assessment important to prevent outbreaks

Dr Andrew D. Turner CChem CSci

Principal Chemist Food Safety Group

Topic Leader Natural Aquatic Toxins

FAO Reference Centre Marine Toxins Advisor

Andrew.turner@cefas.co.uk

Cefas INTERNATIONAL CENTRES OF EXCELLENCE

Food and Agriculture Organization of the United Nations