FAO Reference Centre for Bivalve Sanitation workshop on the development of bivalve production in Africa

8th – 10th July 2025, Nairobi, Kenya

Public hazards associated with bivalve molluscs

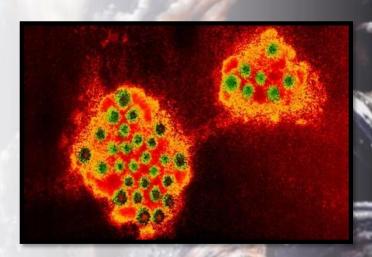
Dr James Lowther and Dr Andrew Turner

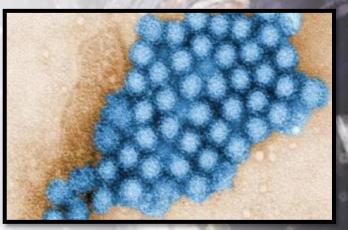




Shellfish and disease risk

- Shellfish are an established route of transmission for a range of important human pathogens and other agents of illness.
 First outbreaks linked to shellfish consumption emerged in the 19th Century
- Filter feeding bivalves (oysters, mussels, clams etc.) very efficient at concentrating microbes, algal toxins and other chemicals. Bivalve shellfish can concentrate microbial pathogens >100 times compared with overlying waters
- Outbreaks can be very large: Hepatitis A outbreak in China in 1988 due to contamination of clams was responsible for almost 300,000 cases – one of the largest foodborne outbreaks ever reported





Types of hazard associated with bivalve shellfish

TYPE OF HAZARD	SOURCES	GUIDANCE
CHEMICAL; e.g. pesticides, heavy metals	Agricultural run-off, industrial discharges etc.	Codex Alimentarius, General Standard for Contaminants and Toxins in Feed and Food, 2009
MICROBIOLOGICAL; i.e. pathogenic bacteria, viruses, parasites	Human sewage, animal faeces, some naturally occurring in seawater	FAO/WHO Technical Guidance for the Development of Sanitation Programmes
BIOTOXINS ; i.e. toxins produced by marine microalgae	Naturally occurring in seawater, blooms	Assessment and management of biotoxin risks in bivalve molluscs, FAO Technical Paper, 2011



Chemical hazards

A variety of different toxic chemicals can accumulate in shellfish

HEAVY METALS

Lead (Pb), Cadmium (Cd), Mercury (Hg), Arsenic (As) etc.

PERSISTENT ORGANIC POLLUTANTS

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins, furans etc.

RADIONUCLIDES

Radioisotopes of Polonium (Po), Lead (Pb), Thorium (Th), Uranium (U) etc.



Chemical hazards

- Toxic chemicals derive from heavy industry, waste disposal, mining, agriculture (pesticides) etc.
- Studies have shown heavy metals and organic pollutants in African shellfish

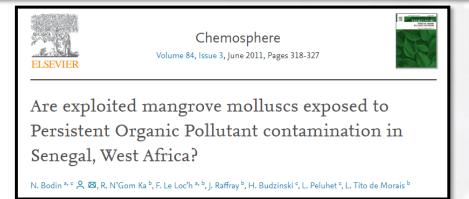


African Journal of Biotechnology Vol. 2 (9), pp. 280-287, September 2003 Available online at http://www.academicjournals.org/AJB ISSN 1684–5315 © 2003 Academic Journals

Full Length Research Paper

Heavy metals concentrations and burden in the bivalves (Anadara (Senilia) senilis, Crassostrea tulipa and Perna perna) from lagoons in Ghana: Model to describe mechanism of accumulation/excretion

Fred A. Otchere





Microbiological hazards

Numerous microbiological pathogens potentially linked to

shellfish consumption

BACTERIA

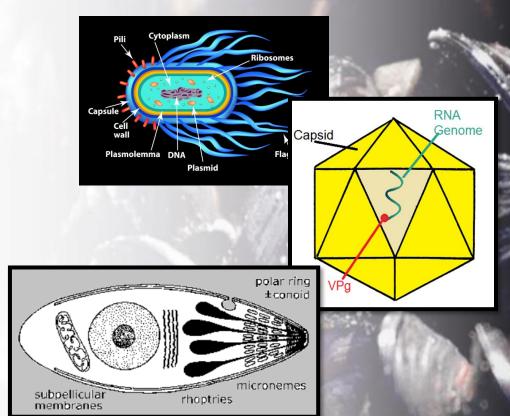
Salmonella spp., Vibrio spp., Campylobacter spp., Listeria monocytogenes

VIRUSES

Norovirus, hepatitis A virus, sapovirus, hepatitis E virus

PARASITES

Giardia intestinalis, Cryptosporidium parvum, Schistosoma spp.



Microbiological hazards

Numerous microbiological pathogens potentially linked to

shellfish consumption

BACTERIA

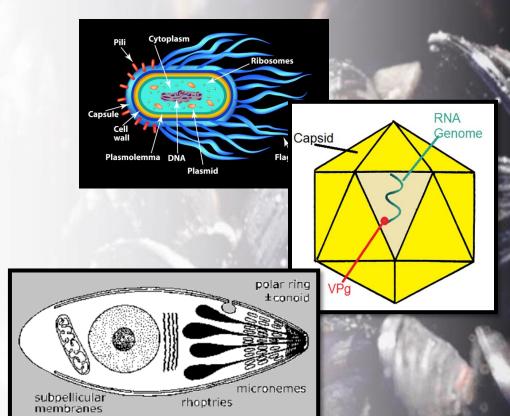
<u>Salmonella spp.</u>, <u>Vibrio spp.</u>, Campylobacter spp., Listeria monocytogenes

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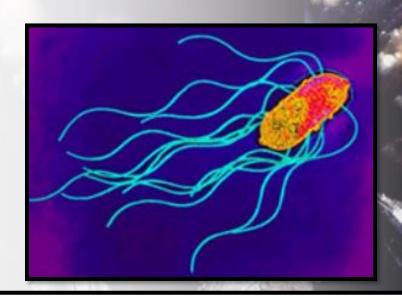
PARASITES

Giardia intestinalis, Cryptosporidium parvum, Schistosoma spp.



Salmonella spp.

- Gram negative bacterium
- Many types (serovars) most types cause gastroenteritis (mild to moderate illness)
- Serovars Typhi and Paratyphi cause enteric fever (severe illness)
- Transmitted in human or animal faeces (depending on serovar)
- First recorded outbreak (serovar Typhi) due to shellfish consumption in 1894



The New York Times

TYPHOID FEVER DUE TO OYSTERS.; Wesleyan University Faculty's Explanation of the Recent Epidemic.

Nov. 14, 1894

Vibrio spp.

- Gram negative bacterium
- Depending on species, causes gastroenteritis (mild to severe illness) or sepsis (severe illness with high mortality in susceptible cases)
- Naturally occurring in marine environment; associated with low salinity, high temperature coastal waters
- Commonest shellfish-related pathogen in e.g. USA (Vibrio parahaemolyticus)
- Other species including *V. cholerae* and *V. vunificus* are frequently linked to shellfish consumption





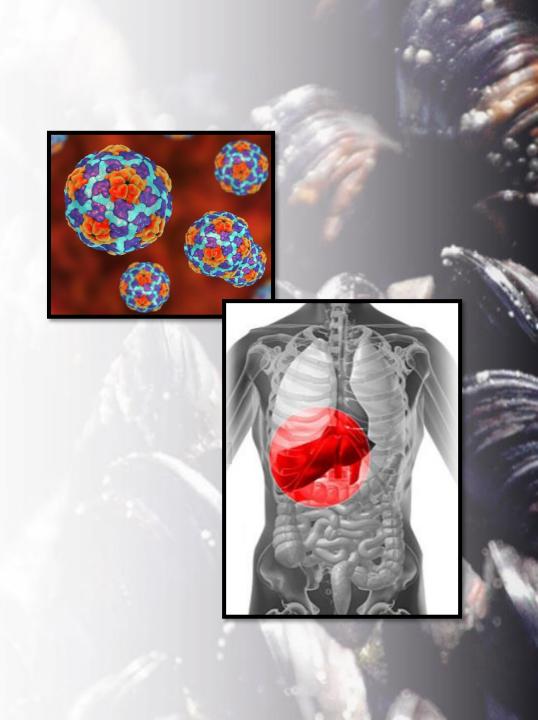
Norovirus

- Single stranded RNA virus
- Causes gastroenteritis (normally mild illness)
- Transmitted in human faeces
- Widespread worldwide
- Commonest shellfish-related pathogen in e.g. Europe
- Highly seasonal occurrence in some regions



Hepatitis A virus

- Single stranded RNA virus
- Causes hepatitis inflammation of the liver (moderate illness)
- Transmitted in human faeces
- Frequency in human populations varies widely across the globe



Shellfish-related pathogens in Africa

 Very few reports in scientific literature of shellfishrelated transmission of microbial pathogens in Africa

Salmonella spp.

High incidence of typhoid fever in Africa

OPEN @ ACCESS Freely available online



Population-Based Incidence of Typhoid Fever in an Urban Informal Settlement and a Rural Area in Kenya: Implications for Typhoid Vaccine Use in Africa

Robert F. Breiman^{1*}, Leonard Cosmas^{1,2}, Henry Njuguna^{1,2}, Allan Audi^{1,2}, Beatrice Olack^{1,2}, John B. Ochieng^{1,2}, Newton Wamola^{1,2}, Godfrey M. Bigogo^{1,2}, George Awiti^{1,2}, Collins W. Tabu³, Heather Burke¹, John Williamson¹, Joseph O. Oundo¹, Eric D. Mintz⁴, Daniel R. Feikin¹

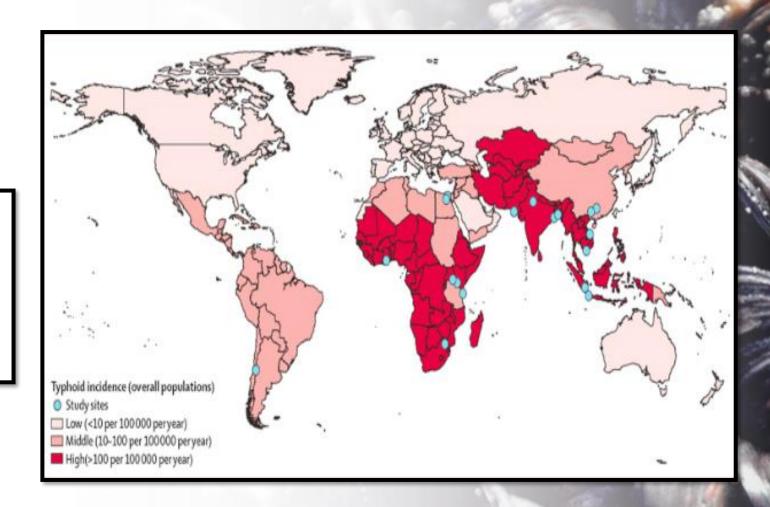
1 Global Disease Detection Division, Kenya Office of the US Centers for Disease Control and Prevention, Nairobi and Kisumu, Kenya, 2 Kenya Medical Research Institute (KEMRI), Nairobi and Kisumu, Kenya, 3 Kenya Ministry of Public Health and Sanitation, Nairobi, Kenya, 4 National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America



RESEARCH ARTICLE

Spatial and temporal heterogeneities of district-level typhoid morbidities in Ghana: A requisite insight for informed public health response

Frank Badu Osei 61*, Alfred Stein1, Sylvester Dodzi Nyadanu2



Vibrio spp.

Vibrio parahaemolyticus recorded in many African countries (including pathogenic strains)

Vibrio spp. detected in seafood in some countries

Journal of Medical Case Reports

CASE REPORT

Open Access

Surveillance and laboratory collaboration in response to an outbreak of *Vibrio* parahaemolyticus, *Plesiomonas shigelloides*, and *Aeromonas hydrophila* in Sekondi-Takoradi, Ghana: a case series

Michael Owusu^{1,7*}, Bernard Nkrumah^{2*}, Ebenezer Kofi Mensah³, Jones Lamptey¹, Godfred Acheampong¹, David Sambian¹, Augustina Sylverken^{7,8}, Shannon Emery⁴, Lucy Maryogo Robinson⁴, Solomon Asante Sefa³, Eric Amoako³, Irene Amedzro³, Slyvester Chinbuah³, Kwame Asante⁴, Yaw Adu-Sarkodie⁵ and David Opare⁶



♠ Foodborne Pathogens and Disease > VOL. 10, NO. 12 | Original Articles

Vibrio cholerae and Vibrio parahaemolyticus Detected in Seafood Products from Senegal

Ignace Coly ⊡, Amy Gassama Sow, Malang Seydi, and Jaime Martinez-Urtaza

Published Online: 21 Nov 2013 | https://doi.org/10.1089/fpd.2013.1523

Norovirus

- Norovirus a common cause of gastroenteritis in Africa
- Small number of studies showing detection of norovirus in shellfish grown in African countries



COLLECTION REVIEW

Norovirus Epidemiology in Africa: A Review

Janet Mans¹*, George E. Armah², A. Duncan Steele³, Maureen B. Taylor¹

- 1 Department of Medical Virology, University of Pretoria, Pretoria, South Africa, 2 Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana, 3 MRC Diarrhoeal Pathogens Research Unit, University of Limpopo, Pretoria, South Africa
- E Current address: Bill and Melinda Gates Foundation, Seattle, Washington, United States of America
- * janet.mans@up.ac.za

Tropical Medicine and International Health

VOLUME 21 NO 1 PP 2-17 JANUARY 2016

Review

Human Norovirus prevalence in Africa: a review of studies from 1990 to 2013

Jean Pierre Kabue¹, Emma Meader², Paul R. Hunter^{2,3} and Natasha Potgieter¹

- 1 Department of Microbiology, School of Mathematical and Natural Sciences, University of Venda, Thohoyandou, RSA 2 School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK
- 3 Department of Environmental Health, Tshwane University of Technology, Pretoria, RSA

Food and Environmental Virology

Norovirus Detection at Oualidia Lagoon, a Moroccan Shellfish Harvesting Area, by Reverse Transcription PCR Analysis

Original Paper | Published: 13 April 2019 Volume 11, pages 268–273, (2019)

N. El Moqri M, F. El Mellouli, N. Hassou, M. Benhafid, N. Abouchoaib & S. Etahiri

Hepatitis A virus

- High prevalence of HAV in Africa
- May be low levels of symptomatic HAV disease due to high population immunity
- Small number of reports showing HAV detection in African shellfish
- Export to low prevalence countries potentially risky e.g. exports from Peru have caused HAV outbreaks in Europe



Food and Environmental Virology (2020) 12:274–277 https://doi.org/10.1007/s12560-020-09432-2

LETTER TO THE EDITOR

Contamination of Clams with Human Norovirus and a Novel Hepatitis A Virus in Cameroon

Patrice Bonny^{1,2,3} · Marion Desdouits¹ · Julien Schaeffer¹ · Pascal Garry¹ · Jean Justin Essia Ngang² · Françoise S. Le Guyader^{1,4}

Shellfish-related pathogens in Africa

- Very few reports in scientific literature of shellfishrelated transmission of microbial pathogens in Africa
- All major shellfish-related pathogens found in the African general population
- Under-reporting probable microbiological risks from shellfish in Africa likely as significant as those in other regions

Methods for pathogen testing

 International Standard Methods for all major shellfish-related pathogens are available

Salmonella spp.	ISO 6579-1	Detection by growth on selective bacteriological media – confirmation using biochemical/serological tests
<i>Vibrio</i> spp.	ISO 21872-1	Detection by growth on selective bacteriological media – confirmation using biochemical/PCR tests
Norovirus and Hepatitis A virus	ISO 15216-1	Quantification using real-time RT- PCR





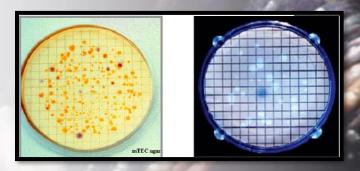


Methods for pathogen testing

- Methods for direct pathogen testing tend to be complex and demanding in terms of laboratory equipment and reagents
 - Method for *Vibrio* spp. requires mutliple incubators at different temperatures, 2 different chromogenic solid media for *Vibrio* isolation, biochemical or PCR reagents to confirm putative *Vibrio* isolates etc.
 - Method for norovirus and HAV (quantitative RT-PCR) requires specialised RNA extraction and PCR reagents, real-time PCR machine etc.
- Normal approach in bivalve sanitation programmes in Europe, North America etc. is to use simpler methods for enumeration of a single faecal indicator organism

Faecal indicator testing

- In European Union reference method is Most Probable Number (MPN) test for *E.coli* in shellfish flesh
- In United States reference method is MPN or membrane filtration test for **faecal coliforms in seawater**
- In all cases a robust monitoring programme requires:-
 - regular taking of samples from all shellfish growing areas
 - rapid transport of samples to laboratories <u>under</u> temperature control
 - testing of samples by competent (accredited)
 microbiology laboratories using appropriate methods



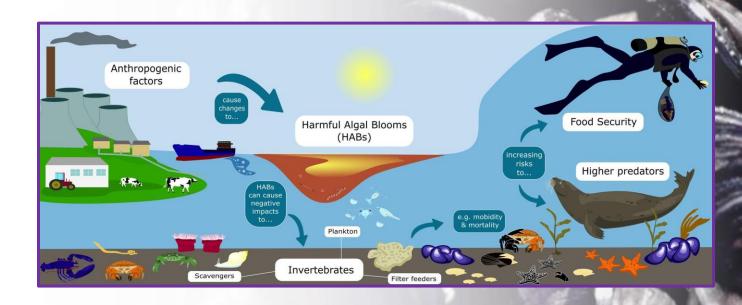




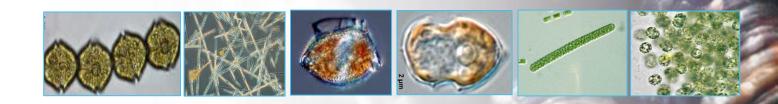
Biotoxin hazards associated with bivalve mollusc consumption: Risks and Responses

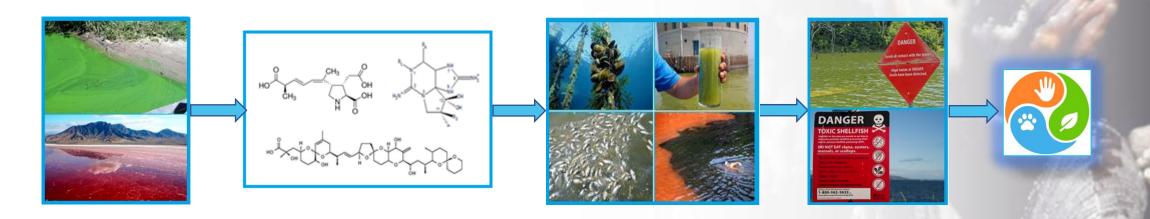
Overview

- Harmful Algal Blooms
- EU regulated marine toxins
- Monitoring programme requirements
- Additional toxins of concern



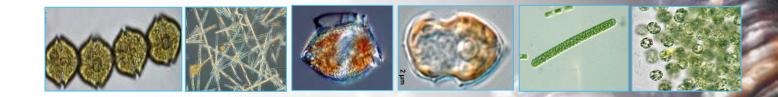
Algal blooms





- Microscopic algae (phytoplankton) can periodically form blooms
- Some species can naturally produce harmful toxins
- · Toxins can affect water quality or accumulate in shellfish or fish
- Toxins can affect human health, animal health, environmental 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
- Very low levels can cause toxicity

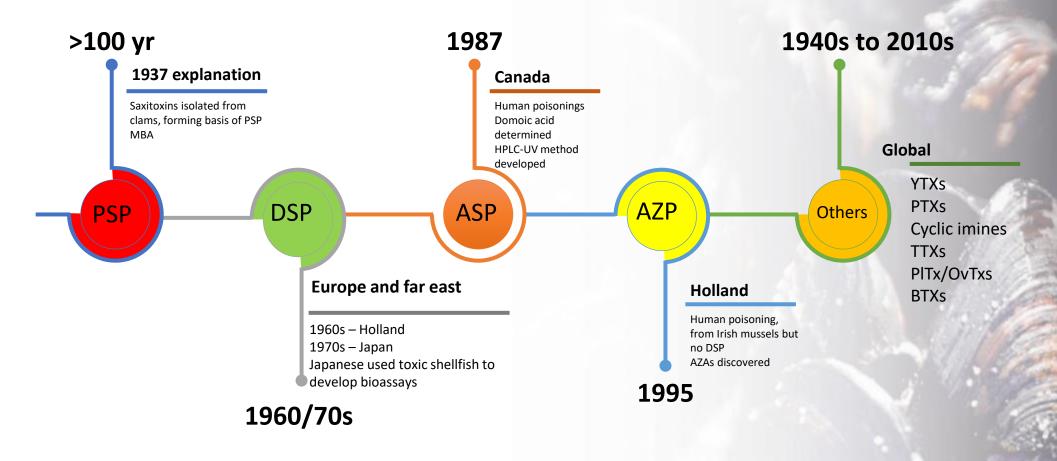
Animal Health

- 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**

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);

EU regulated marine biotoxins

Why are these important?

- In-country consumption of hazardous seafood
- Exporting fishery products to EU legal requirements



II
(Non-legislative acts)

REGULATIONS

COMMISSION DELEGATED REGULATION (EU) 2019/624
of 8 February 2019

concerning specific rules for the performance of official controls on the production of meat and for production and relaying areas of live bivalve molluses in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council

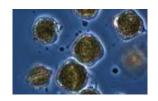
 Exporting to other regions (e.g. US/Can, Aus/NZ) –some differences in legal/practical requirements

Paralytic Shellfish Poisoning (PSP) - Saxitoxins

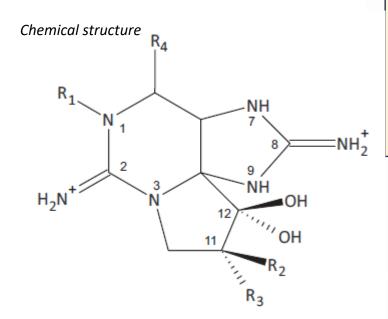
Source phytoplankton







- Alexandrium sp.
- Gymnodinium catenatum
- Pyrodinium bahamense (all dinoflagellates)



- >57 analogues
- Wide ranging toxicities
- Water soluble
- 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 800 μg STX eq/kg maximum permitted level

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

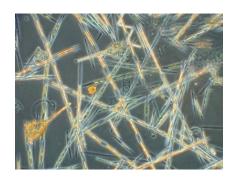
- Notable changes over time
- Increased/improved monitoring and detection capabilities AND/OR
- Global expansion of HABs
 - Anthropogenic inputs
 - Environmental/climatic change
 - Water/shellfish stock movements



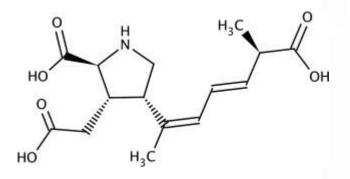


Amnesic Shellfish Poisoning (ASP) – Domoic acid(s)

Source phytoplankton



 Pseudo-nitzchia sp. (diatom)



- 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 maximum permitted limit

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
- Australia / New Zealand
- Less in tropical/sub-tropical areas

Amnesic Shellfish Poisoning



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

Diarrhetic Shellfish Poisoning (DSP) - Lipophilic Toxins

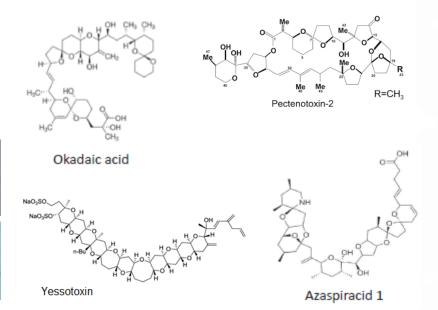
Source phytoplankton







- · Dinophysis sp.
- Prorocentrum sp.
- Azadinium sp.
- Amphidoma sp.
- Lingolodinium sp.
 All dinoflagellates



- 11 Okadaic acid (OA) analogues
- >60 Azaspiracids (AZAs)
- Multiple yessotoxins (YTXs)
- Pectenotoxins (PTXs) now deregulated

Human health

- DSP = Most common shellfish toxin poisoning
- 2001-2015 ~1200 reported intoxications globally
- Actual impact much higher, lack of clear symptoms
- Azaspiracid shellfish poisoning (AZP) most recent ~200 intoxications to date
- YTXs/PTXs no known acute health impacts

Animal Health

Yessotoxin impact on mass invertebrate mortalities

Inhibition of protein phosphatases Ecosystem Health

Trophic impacts following die-offs

• Intense gastric symptoms (temporary), tumour promotion

Global distribution of DSP

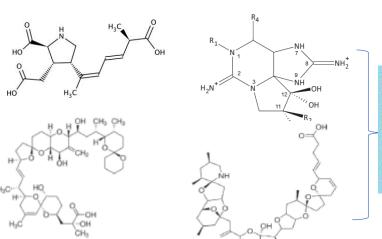


Global distribution Dinophysis toxin detection (ICES-IOC HAEDAT, 2014)



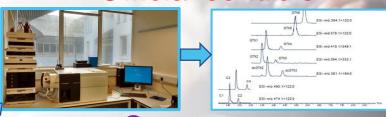
Courtesy of U.S. National Office for Harmful Algal Blooms (https://hab.whoi.edu/maps/regions-world-distribution)

Risk management through monitoring





Official controls



Competent Authority

End Product Tests



Industry











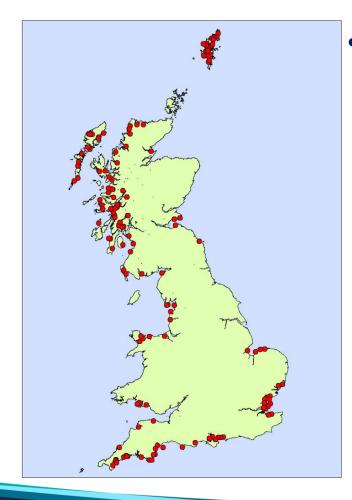




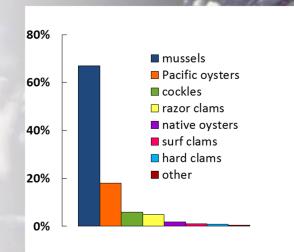




Official control monitoring



- OC phytoplankton and biotoxin monitoring programme
 - Samples are collected from predetermined monitoring points (weeklymonthly)
 - From designated shellfish harvesting areas
 - Wide variety of shellfish species
 - Toxins results reported 1 day after receipt
 - Phytoplankton 2 days after receipt







What happens if toxins/harmful plankton is detected

Shellfish flesh – EU regulatory limits

PSP: 800 µg/kg flesh

ASP: 20 mg/kg flesh

OA/DTXs: 160 µg/kg flesh

AZAs: 160 µg/kg flesh

YTXs: 3.75 mg/kg flesh



If regulatory limit exceeded

close area/recall product

Continue monitoring -- 2 negative results allow reopening of area

Water - Trigger levels (UK example)

PSP producing algae: Presence (40 cells/L)

ASP prod. algae: e.g. 150,000 cells/L

DSP prod. algae: 100 cells/L



If trigger levels exceeded

Increase monitoring

Toxin testing – OC process overview

- Representative samples collected
- Shipped to laboratory in cool boxes with ice packs and foam padding
- Live shellfish arrive at testing lab
- Homogenisation (blending)
 - >50 grams tissue; minimum 10 animals
- Weigh into three samples (ASP, LT, PSP)

All tests involve

- Solvent extraction (to remove toxins from shellfish tissue)
- Clean-up (chemical and/or physical)
- Analysis overnight on instrument
 - Separation
 - Detection
- Reporting results to CA



Regulatory testing methods - toxins

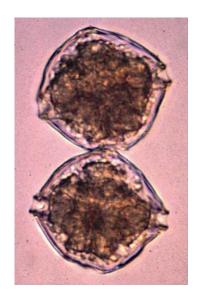
- ASP Liquid Chromatography with Ultraviolet Detection (LC-UV)
- PSP Pre-column oxidation LC with Fluorescence
 Detection (pre-cox LC-FLD)
- LTs LC with tandem mass spectrometry (LC-MS/MS)

Advantages	Disadvantages
Thoroughly validated	Intensive work, highly trained staff
Highly specific (targeted)	Overnight run
Accurate concentration assessment	Expensive purchase & maintenance
Reproducible	Specific targets – other toxins could be missed
Ethically sound	Currently no toxicity screen

Methods defined in EC 2017/625 – Annex V



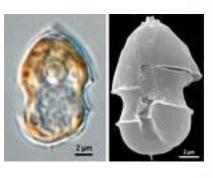
Water testing - Toxin-producing phytoplankton



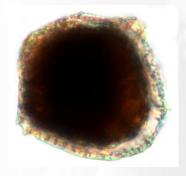
Alexandrium sp.



Dinophysis sp.



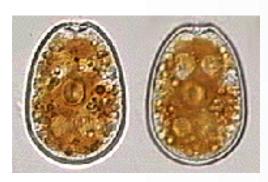
Azadinium spinosum



Protoceratium reticulatum

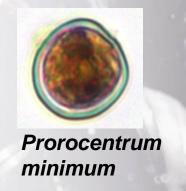


Lingulodinium polyedrum



Prorocentrum lima

Pseudonitzschia sp.







Water sample collection

Samples must be <u>representative</u> of the algal community in the water body being sampled

- Sampling methods
 - Tube / pole samplers
 - Nets
 - Surface water
- Cells are easily damaged sample must be fixed as soon as possible after collection to keep cell integrity



Phytoplankton testing

- Representative samples collected
 - Fixed with Lugol's iodine
- Shipped to laboratory
- Homogenisation (mixing)
- Dispense into Utermöhl chambers
 - Leave for 24hours to settle
- Analysis by base plate count of algal taxa
- Qualitative species assessment
- Quantitative cell density







End product testing

ELISA/PP2A





LFA



ELISA/Functional	Lateral Flow Assays (LFA)
Multiple suppliers e.g. ZeuLab, Abraxis, Beacon, Gold Diagnostics, R-Biopharm	Two suppliers – Sensoreal (Canada) and AquaBC (Chile)
More expensive	Cheapest monitoring approach
Plate readers, multi-channel pipettes, incubators	Pipettes + scanner
Higher level of personnel training	Easy to use by non-scientists
Require laboratory to use	Can operate in field

4 2004 EN

Official Journal of the European Union

T 155/206

REGULATION (EC) No 854/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004

laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption

Food businesses required under EU law to ensure that shellfish placed on the market are safe for consumption and do not exceed the MPLs stipulated in the EC regulations



Requirements

OC testing

Laboratory

- LC-MS/MS and HPLC instruments + PCs
- Reliable electrical power
- Maintenance servicing
- Apparatus and consumables
- Chemicals and reagents
- Toxin standards
- Validation and accreditation

Personnel

- Shellfish Sampling Officers
- Trained chemists and phycologists
- Health and Safety
- Quality Managers
- Project Managers
- Reporting & Customer service

Samples

- Access to representative samples (live molluscs)
- Effective transportation

End Product Testing

"Laboratory"

- Small power supply
- Basic consumables (pipettes)
- Basic reagents
- Ability to import tests

Personnel

- Samplers
- Operative (basic training only)
- Knowledge to respond to results

Samples

- Access to representative samples (live molluscs)
- Effective transportation
- Can be done on-site at farm



Brevetoxins

Neurotoxic Shellfish Poisoning



$$\begin{array}{c} \text{Me} \\ \text{Me} \\ \text{B} \\ \text{C} \\ \text{D} \\ \text{E} \\ \text{O} \\ \text{A} \\ \text{30} \\ \text{O} \\ \text{E} \\ \text{O} \\ \text{A} \\ \text{A} \\ \text{O} \\ \text{A} \\ \text{A} \\ \text{O} \\ \text{A} \\$$

- Brevetoxins include both algal and shellfish metabolites
- BTX B2, B4, B5; PbTx2, PbTx3, S desoxy BTX B2



The classic "Red Tide"

Brevetoxins from Karenia brevis

Until recently associated with USA and NZ event

Now found in parts of Mediterranean Sea

Brevetoxins

- Due to regular occurrence in parts of USA, brevetoxins are regulated
- A regulatory limit of 800 μg BTX-2 equivalents is established by US-FDA
- Brevetoxins have been detected in Europe (France) but are not yet regulated in the region

- Brevetoxins include both algal and shellfish metabolites
- BTX B2, B4, B5; PbTx2, PbTx3, S desoxy BTX B2



Toxins summary

- Three main regulated groups PSP, ASP, LTs including DSP
- Importance of both OC testing and EPT for risk management
- Processes are complex and expensive
- Setting up toxin/phytoplankton monitoring & risk management programme is complex, time-consuming and expensive

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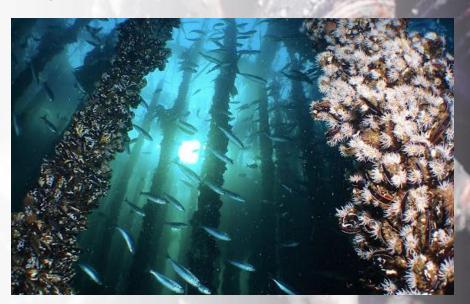
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Summary – all hazards

- Wide variety of microbiological, biotoxin and chemical hazards associated with shellfish consumption
- Microbiological hazards include bacteria (Salmonella, Vibrio), viruses (norovirus, hepatitis A virus), parasites
- Toxins ASP, PSP, LTs (and BTXs)
- Chemicals Metals, Radioisotopes, Organic compounds
- Evidence of shellfish-related hazards in several African countries limited but expanding knowledge
- Risks posed by different hazards may depend on the characteristics of the growing area

KINGDOM Theme = "One Health"

International Conference on Molluscan Shellfish Safety

16th ICMSS

September 6th- 11th 2026

The Venue
'University of Exeter'
Southwest England













