



MICROBIOLOGICAL, BIOTOXIN AND CHEMICAL TESTING FOR BIVALVE MOLLUSC PRODUCTION

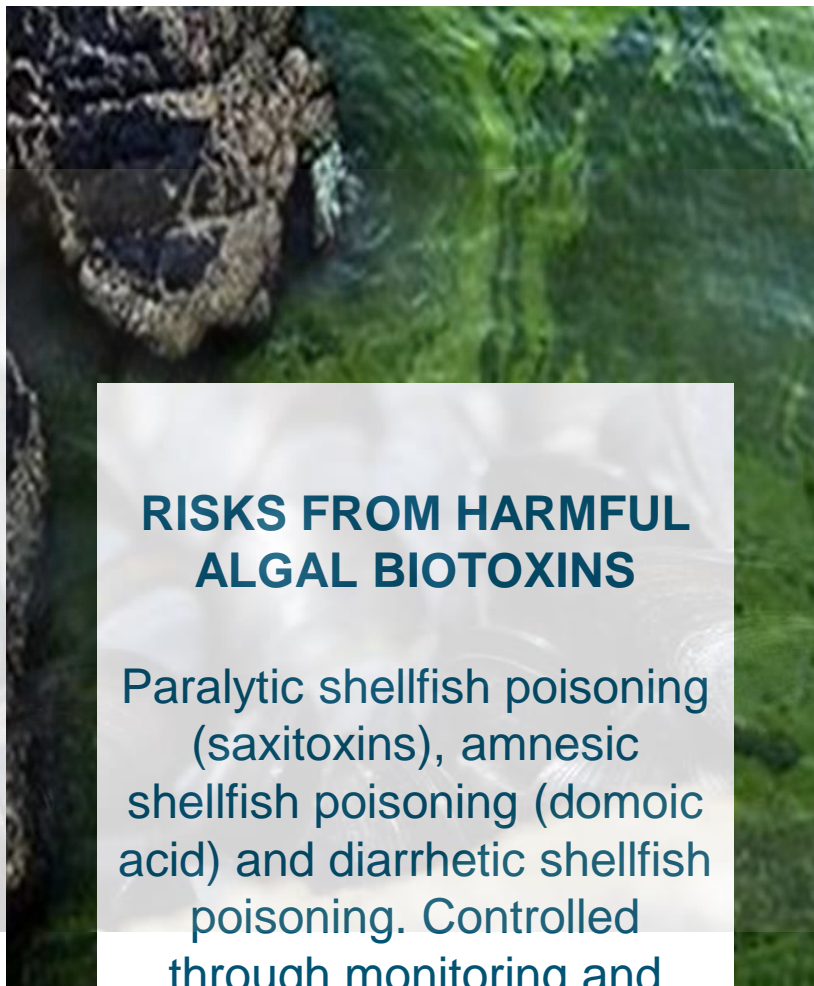
FAMILIARISATION AND TRAINING - DEPARTMENT OF FISHERIES - BANGLADESH
21st - 22nd SEPTEMBER 2022

MAJOR RECOGNISED REGULATED RISKS ASSOCIATED WITH BIVALVE MOLLUSCS IN THE EU



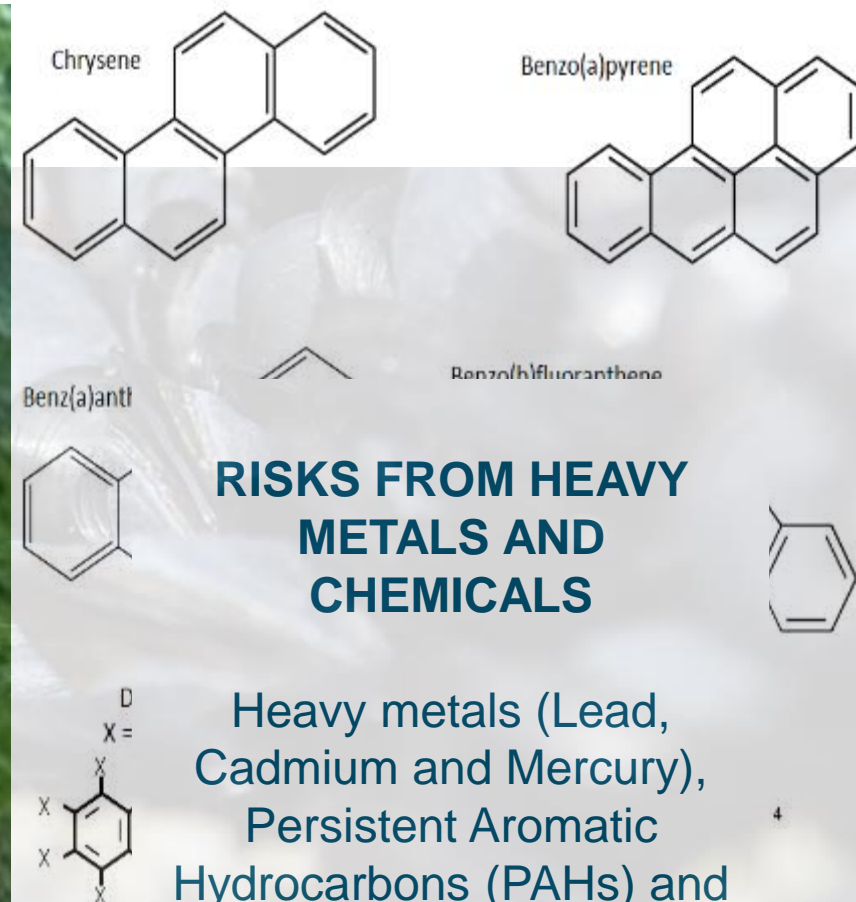
HUMAN (and animal) DERIVED PATHOGENS FROM FAECAL WASTE

Salmonella spp. other faecally transmitted pathogens (norovirus, hepatitis A virus). Controlled through faecal indicator monitoring, area classification and end product standards



RISKS FROM HARMFUL ALGAL BIOTOXINS

Paralytic shellfish poisoning (saxitoxins), amnesic shellfish poisoning (domoic acid) and diarrhetic shellfish poisoning. Controlled through monitoring and harvesting restrictions



RISKS FROM HEAVY METALS AND CHEMICALS

Heavy metals (Lead, Cadmium and Mercury), Persistent Aromatic Hydrocarbons (PAHs) and dioxins. Controlled through harvesting restrictions



Methodological approach will be informed by the Risk Profile e.g., intended markets, use, identified hazards

Regulatory and non-regulatory methods applied for EU markets

Require testing of bivalve flesh and intervalvular fluid

Regulatory methods recommended for United States exports

Require testing of bacterial indicators in water

May be required depending on risk

MATRIX	TARGET ORGANISM	METHOD
Bivalve molluscs	Sample preparation for all bacteriological methods	ISO 6887-3
	Preparation of dilutions of homogenized samples for all bacteriological methods	ISO 6887-1
	<i>E. coli</i>	ISO 16649-3 (5 tube format)
	MSC	EURL generic protocol (EURL 2007) FDA MSC Method
	<i>Salmonella</i> spp. (detection)	ISO 6579-1
	<i>Salmonella</i> spp. (quantification)	ISO 6579-3
	Pathogenic vibrios	See FAO/WHO (2016)
	Hepatitis A virus and norovirus (quantification)	ISO/TS 15216-1
	Hepatitis A virus and norovirus (qualitative detection)	ISO/TS 15216-2
	Water	Faecal coliforms and presumptive <i>E. coli</i> by membrane filtration
Faecal coliforms and presumptive <i>E. coli</i> by Most Probable Number (MPN)		ISO 9308-2
MSC		ISO 10705-1
Standard Methods for the Examination of Water and Wastewater (APHA, 1985)		APHA
Bivalve molluscs	<i>Vibrio parahaemolyticus</i> , <i>V. vulnificus</i> , <i>V. cholerae</i>	e.g. ISO 21872-1, BAM chapter 9 or equivalent (MPN format)

BACTERIAL INDICATORS – ‘NON PATHOGENS THAT CAN INDICATE RISK/LIKELIHOOD OF PATHOGENS ASSOCIATED WITH (ANIMAL OR HUMAN FAECAL) CONTAMINATION’

Typically those used in food safety or process controls are:

Total coliforms (TC) - Very broad taxonomic group, found in soil, animal/human guts

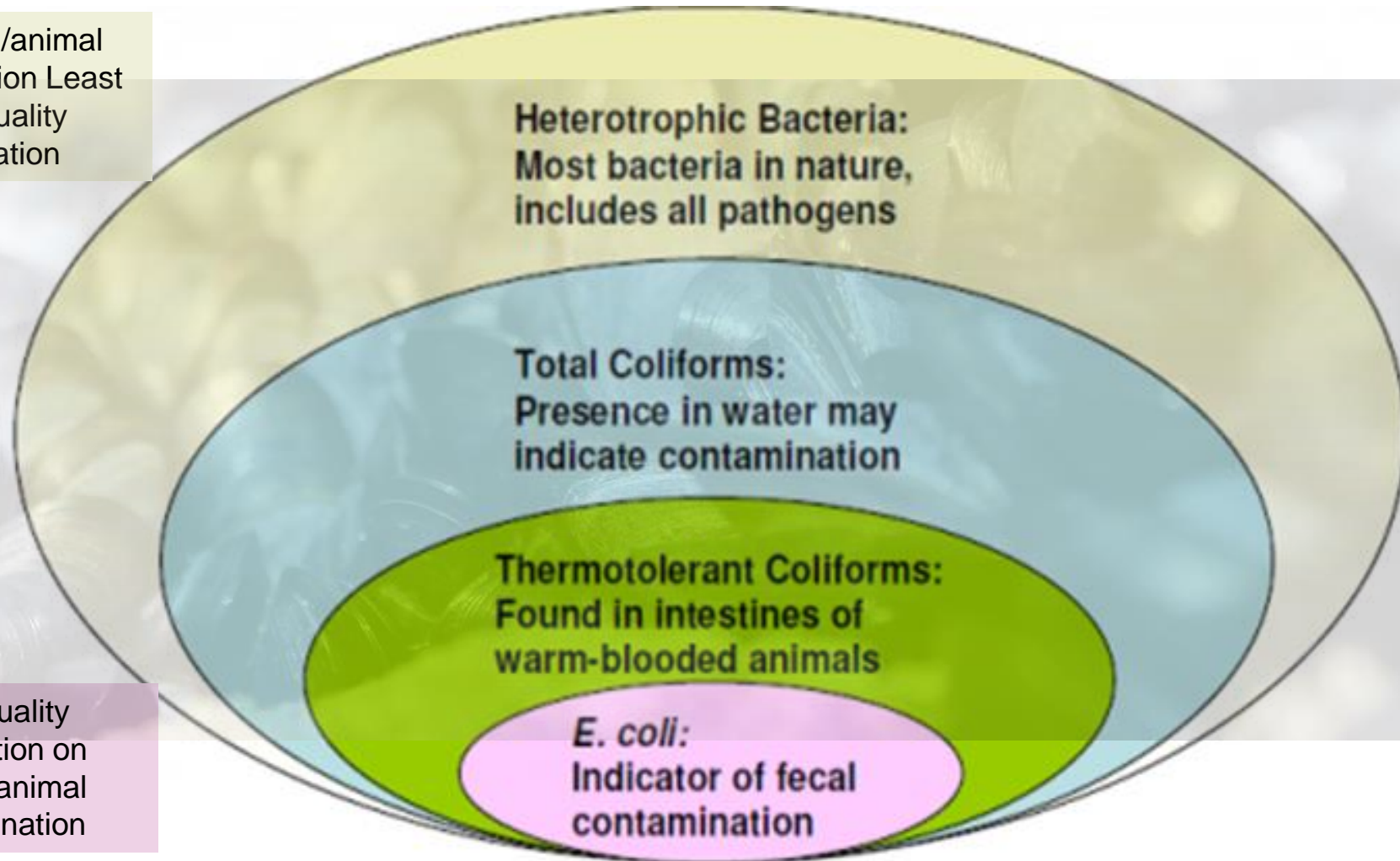
Thermotolerant (Faecal) coliforms (FC) – smaller group but not always derived from warm blooded animals

***E. coli* (non enterotoxigenic)** – presence in foods always indicates contamination with faecal contamination

on human/animal contamination Least good quality information



Best quality information on human/animal contamination



FAECAL INDICATOR: EU *E. coli* reference method for bivalve molluscs

ISO 16649-3:2015 -
Microbiology of the food chain
-- Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*

Required in regulation for growing area **monitoring and classification (primary production)** and **end-product standard** compliance for export into European markets



Bivalve sample preparation (ISO 6887-3)



Mineral modified glutamate incubation for recovery of marine stressed cells

TABLE 1: *E. coli* Most Probable Number (MPN)

MPN of organisms: table for multiple tube methods using 5 x 1g, 5 x 0.1g, 5 x 0.01g.

1g	0.1g	0.01g	MPN/100g	Category
0	0	0	<18 ¹	1
0	1	0	18	1
1	0	0	20	1
1	0	1	40	2
1	1	0	40	1
1	2	0	61	2
2	0	0	45	1
2	0	1	68	2
2	1	0	68	1
2	1	1	92	2
2	2	0	93	1
3	0	0	78	1

PATHOGEN*: ISO15216-1 method for quantitation of norovirus (NoV) and hepatitis A virus (HAV) in bivalve molluscs

NoV and HAV cannot be reliably cultivated from foodstuff.

Realtime reverse transcriptase (RT) polymerase chain reaction (PCR) method to quantify these RNA viruses

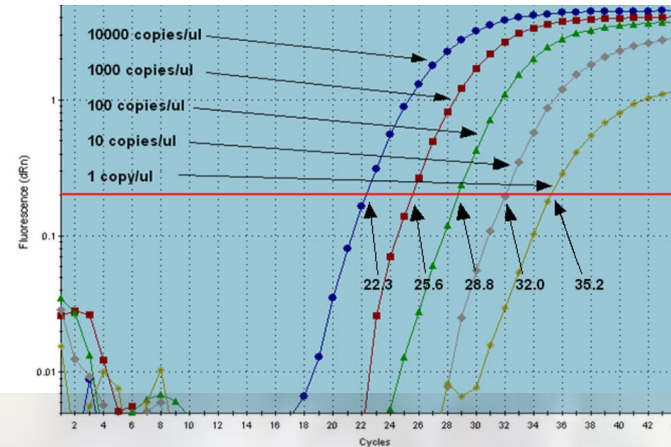
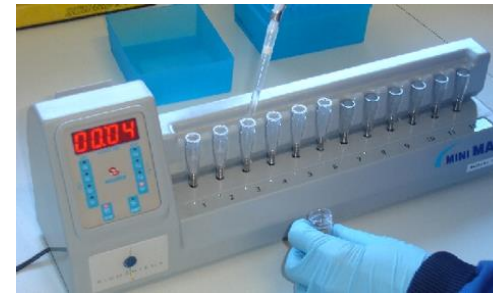
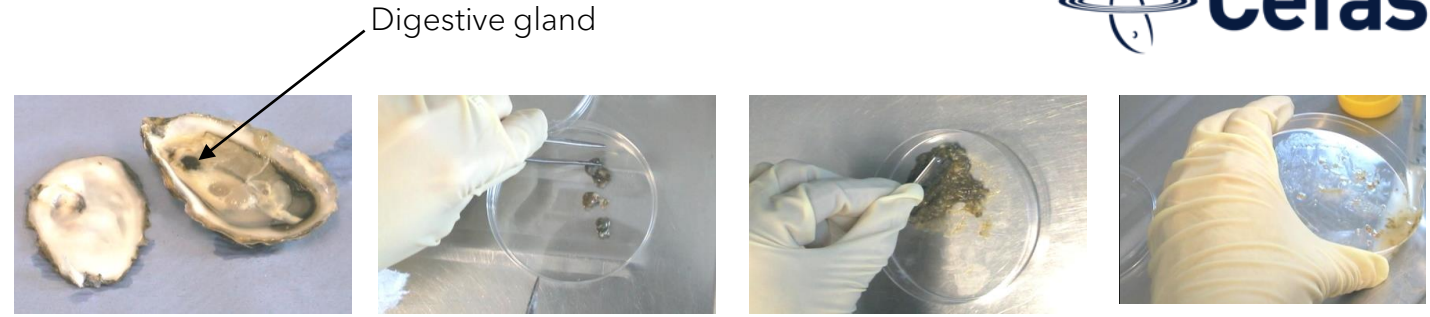
Three steps

1. Virus extraction
2. RNA extraction and amplification
3. Detection and quantitation

Virus extraction

RNA extraction and amplification

Detection and quantitation



Results expressed as genome copies per g of digestive gland

*Only a very few countries (excluding EU, US, Japan, Canada) require NoV or HAV testing for routine imports. Currently the only pathogen microbiological criteria required in EU Regulation for bivalve molluscs placed on the market raw or frozen is *Salmonella* (ISO 6579-1)

Proficiency testing (PT)

PT is used to assess and verify lab's test results

Regular participation,

Demonstrates a lab's commitment to maintaining and improving performance

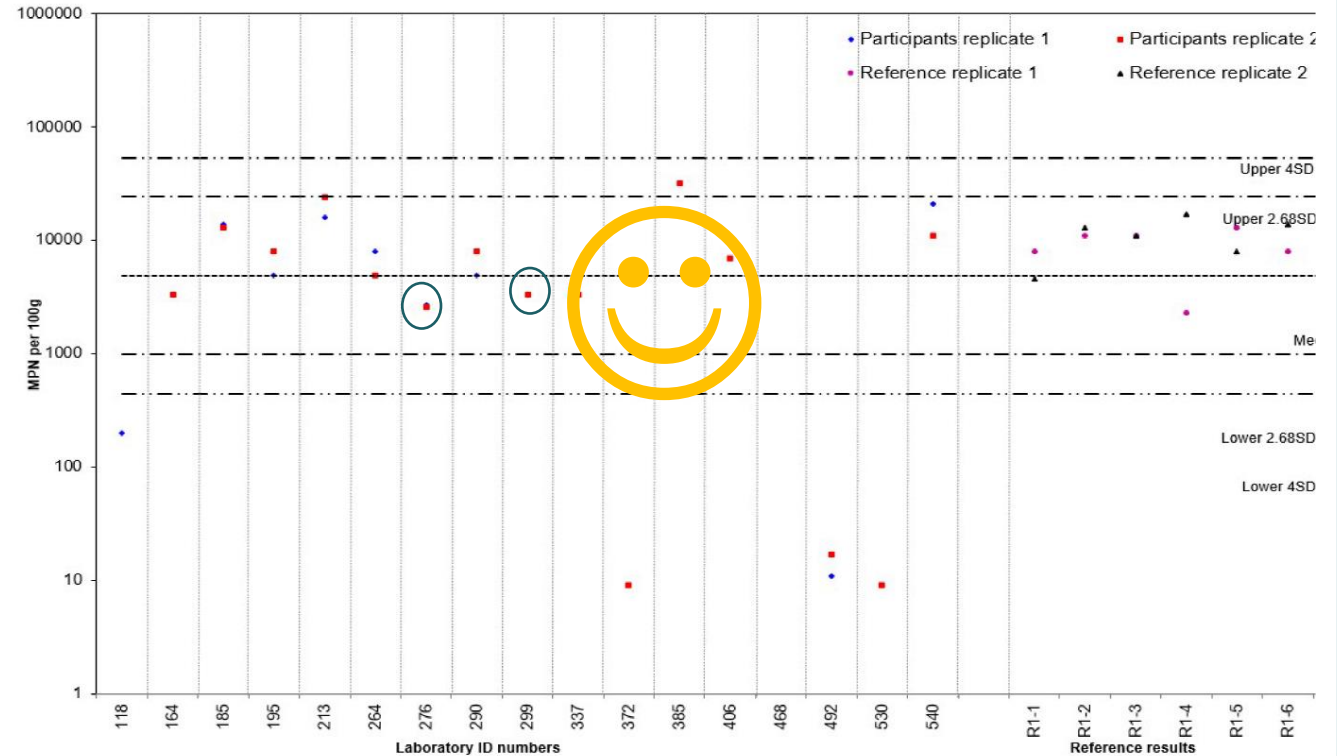
Helps to provide proof of competence for audit (accreditation, trade etc)

Helps to build capability in new methods

Can be used in staff training

Can generate data for verification of methods, analysis of trends and measurement of uncertainty etc

Figure 1: *E. coli* in shellfish - Sample 1

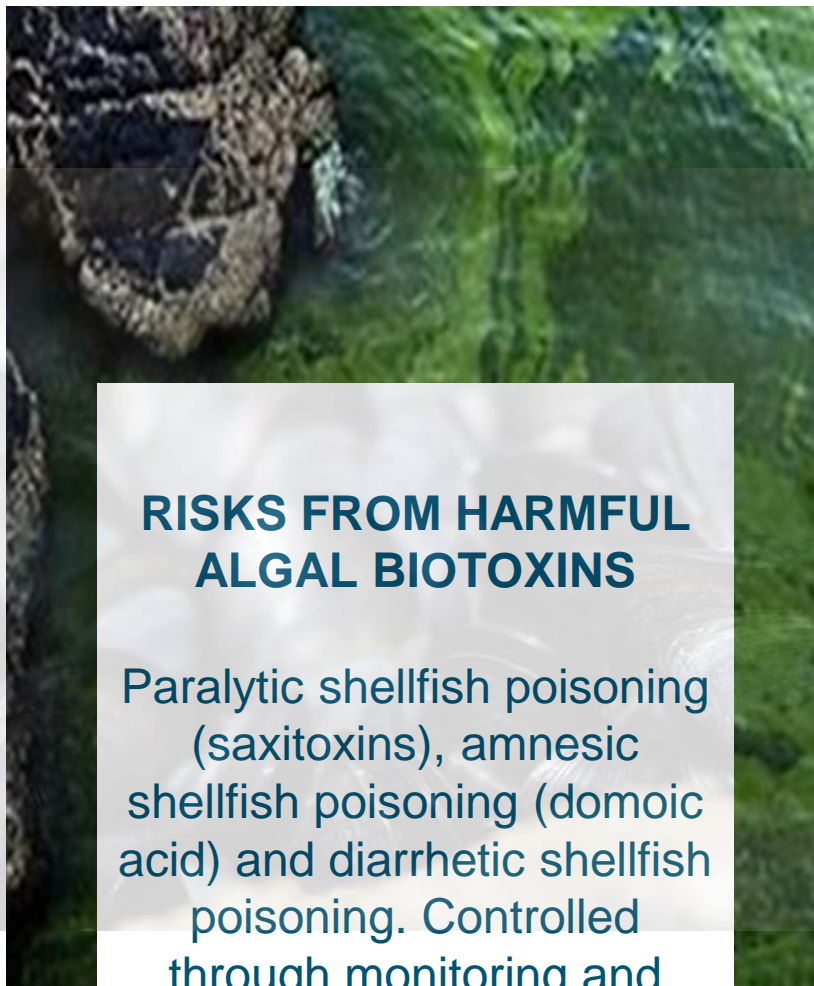


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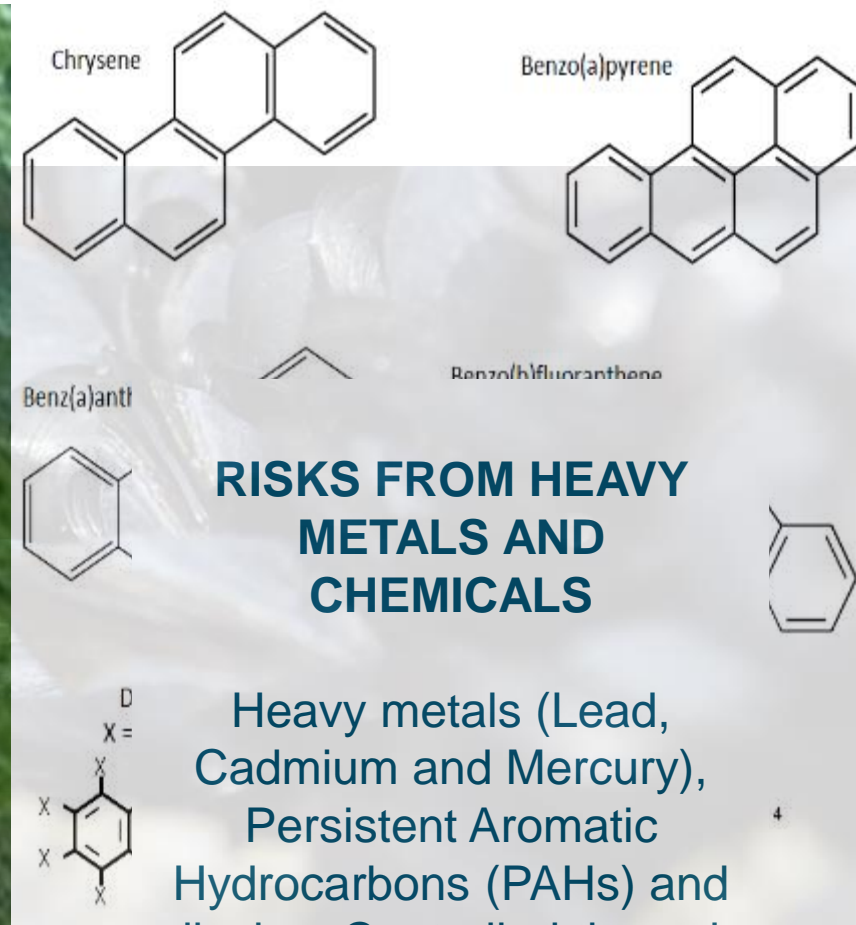
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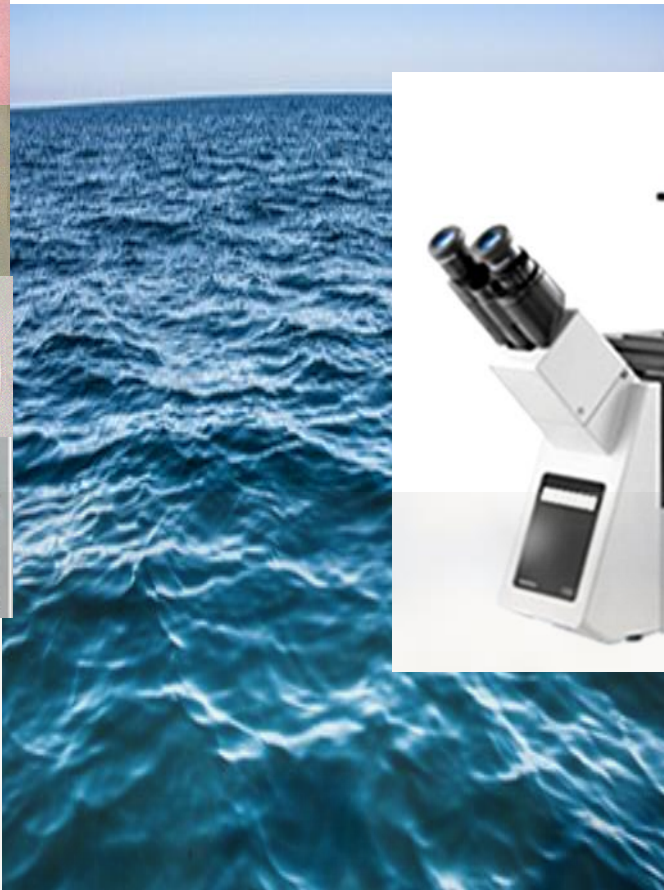
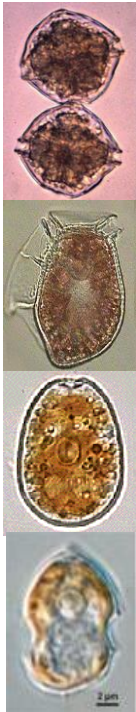
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METHODS FOR CONTROLLING ALGAL BIOTOXINS

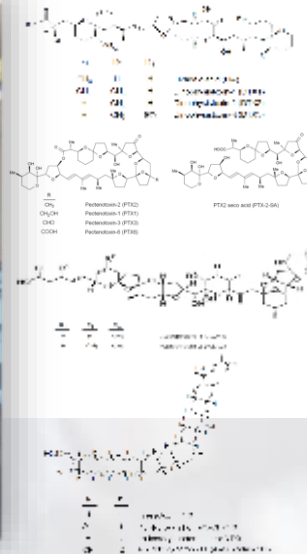
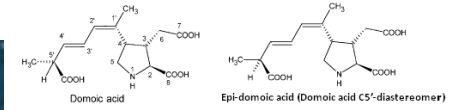
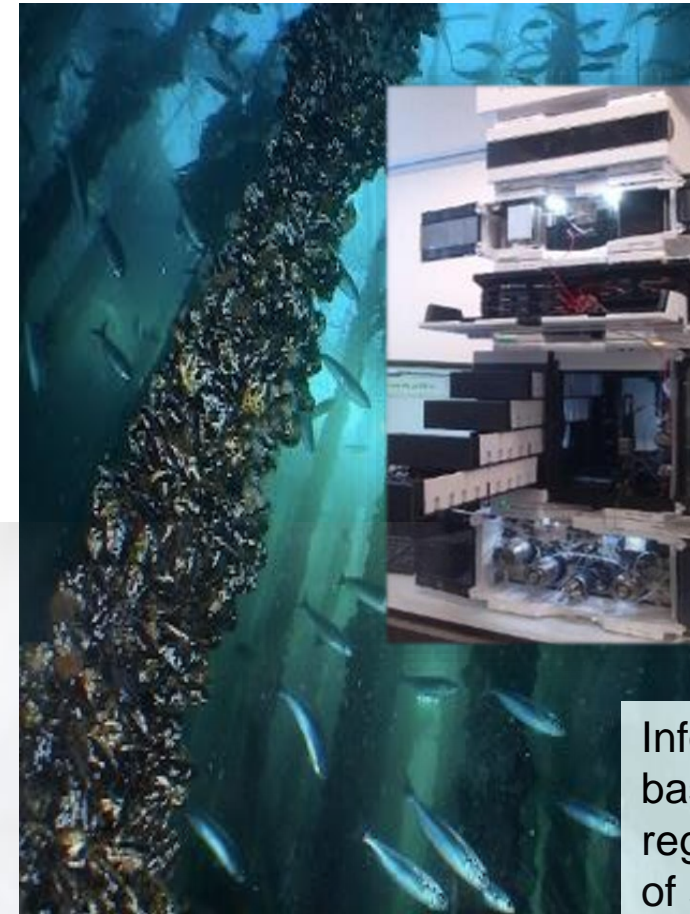


Phytoplankton monitoring in water – using light microscopy



Early warning, targets additional toxin testing

Toxin monitoring in bivalve shellfish detection using instrumental methods (PSP – LC-UV, ASP – LC-FLD [Lawrence method], DSP (lipophilic toxins) LC- tandem MS)



Inform closures based on regulatory limits of specific toxins

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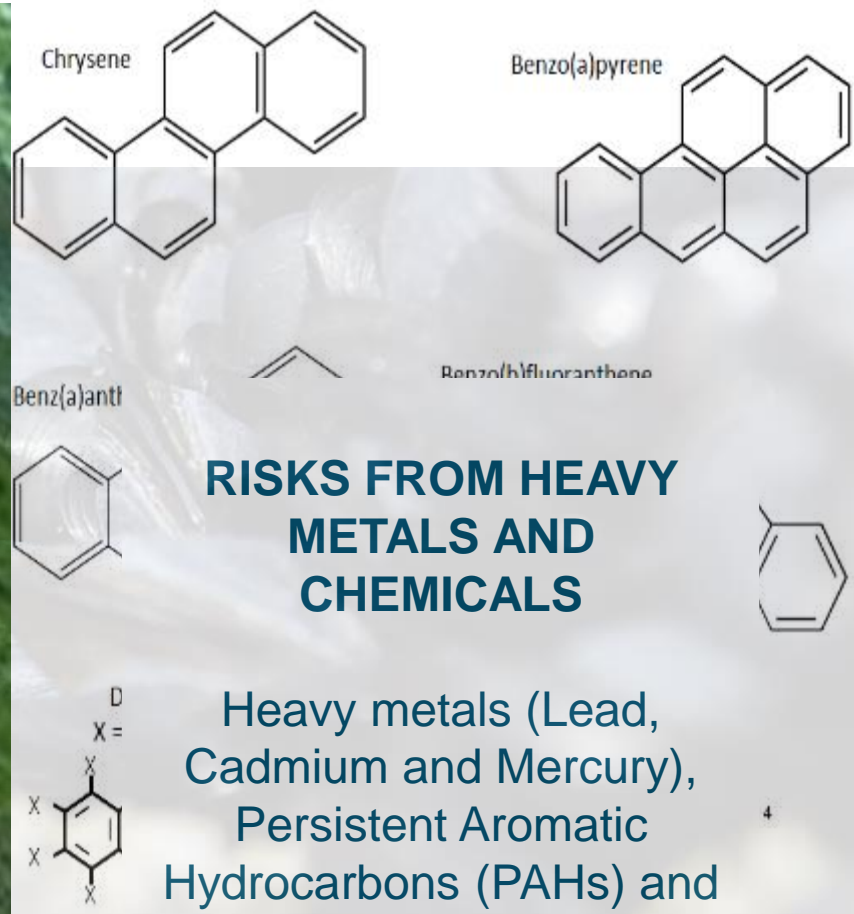
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CHEMICAL CONTAMINANTS

Heavy metals Pb, Cd, Hg (by ICPMS)

PAHs, 4 compounds (by HRGC-LRMS)

Dioxins and dioxin-like PCBs (all organochlorines) (by HRGC-HRMS)

EU limits

Metals

Lead (Pb) - 1.5 mg/kg

Cadmium (Cd) - 1.0 mg/kg

Mercury (Hg) - 0.50 mg/kg

PAHs

5.0 µg/kg for Benzo(a)pyrene

30.0 µg/kg for sum of 4 PAHs:

Benzo(a)pyrene

Benz(a)anthracene

Benzo(b)fluoranthene


Chrysene

Dioxins

Sum of dioxins (WHOPCDD/F-TEQ) = 3.5 pg/g

Sum of dioxins and dioxin-like PCBs
(WHOPCDD/F-PCB-TEQ) = 6.5 pg/g





MICROBIOLOGICAL, BIOTOXIN AND CHEMICAL TESTING FOR BIVALVE MOLLUSC PRODUCTION

SUMMARY



- ❖ In most countries **microbiological, biotoxin and chemical** testing requirements are set out in **regulation** and identified as **the major risks**
- ❖ Risks from **pathogens derived from human or animal faecal** wastes are generally controlled by **bacterial indicator organisms** and standards in **primary production**
- ❖ Recent advances in standardised molecular technology have enabled an ISO standard for main **viruses of concern** which is being used in a few places to detect **NoV and HAV**
- ❖ **Phytoplankton monitoring** gives **early warning** for potential harmful toxic algal species
- ❖ **Toxins** are monitored using **instrumental methods** and **closures** based on **levels of toxins** in bivalve tissues