Food Safety
AUTHORITY OF IRELAND

Report of the Scientific Committee
of the Food Safety Authority of Ireland

The Occurrence of Marine Biotoxins and Risk of Exposure to Seafood Consumers in Ireland

Report of the Scientific Committee
of the Food Safety Authority of Ireland

The Occurrence of Marine Biotoxins and Risk of Exposure to Seafood Consumers in Ireland

Published by:
Food Safety Authority of Ireland
Abbey Court, Lower Abbey St
Dublin 1
DO1 W2H4

Tel: +353 1 8171300
info@fsai.ie
www.fsai.ie

ISBN: 978-1-910348-09-3

The Occurrence of Marine Biotoxins and Risk of Exposure to Seafood Consumers in Ireland

CONTENTS

EXECUTIVE SUMMARY

Vigilance in ensuring the safety of Irish shellfish is of paramount importance and continuous monitoring of shellfish produce for the presence of marine biotoxins is essential to reduce the risk to the consumer. Ireland has a monitoring system in place which can provide predictions of toxin increases and limited forecasting but due to the complexities associated with marine biotoxin formation, regulators face many challenges.

This report describes the risks posed by commonly encountered and novel, or emerging toxins, and describes the monitoring regimes for harmful algal blooms and for marine biotoxins in shellfish currently in place, aimed at controlling these risks. The results of monitoring programmes for the period 2011 to 2013 are presented and a regulatory maximum level exposure assessment scenario for consumers of shellfish in Ireland is provided.

In general, the existing monitoring programmes and regulation of production in shellfish producing areas provide adequate protection against outbreaks of shellfish toxicity among consumers of shellfish in Ireland. However, the continuing success of this programme depends upon further research and monitoring to identify alterations in the geographical or temporal distribution of existing harmful algal blooms or the emergence of novel toxin-producing harmful algal blooms in Irish shellfish producing areas.

Recommendations are made relating to research, regulation and enhanced monitoring. In the area of research, the following issues should be addressed: (a) assessments to identify appropriate indicator species for amnesic shellfish poisoning, diarrhetic shellfish poisoning, paralytic shellfish poisoning and azaspiracid shellfish poisoning toxins (b) assessment of the potential impacts of a changing environment leading to range expansion or alien introduction of non-native harmful algal blooms (c) the obtaining of chronic and sub-chronic toxicity data for shellfish toxins (d) harmonisation of sample pre-treatment practices before the actual analysis of lipophilic marine biotoxins (e) the undertaking of a targeted survey of shellfish consumption in Ireland to verify consumer exposure to shellfish toxins derived in this report and to support the indication that shellfish portion sizes in Ireland are considerably lower than the large portion size of 400g used by the European Food Safety Authority (EFSA).

In the area of regulation, the following issues should be addressed: (a) continued investigation into the occurrence and toxicity of azaspiracid analogues other than azaspiracid 1-3 is essential and legislation needs to be amended to include them, where warranted; (b) the need for toxicity and epidemiological data on pectenotoxins to evaluate the proposed de-regulation of pectenotoxins; (c) the collection of long term data for new and emerging toxins and (d) regulations (and mitigation strategies) put in place to protect consumers, where warranted.

In the area of monitoring, the following issues should be addressed: (a) harmful algal bloom development should be monitored on a local, individual production area scale to establish long term baseline data; (b) levels of paralytic shellfish poisoning toxins in areas where previously not encountered should be closely monitored; (c) monitoring and control of imported fish is recommended for certain biotoxins, such as tetrodotoxin and ciguatoxins.

GLOSSARY

***Alexandrium* spp.:** Phytoplankton species associated with paralytic shellfish poisoning

AOAC: Association of analytical communities

ARfD: Acute Reference Dose

ASP: Amnesic Shellfish Poisoning

AZA: Azaspiracid

AZAs: Azaspiracids AZA 1-33

AZP: Azaspiracid Shellfish Poisoning (part of the lipophilic group)

BIM: An Bord Iascaigh Mhara (the Irish Sea-Fisheries Board)

Competent authority: An authority which is competent to carry out checks, as defined by EU Legislation Code of Practice

CFP: Ciguatera Fish Poisoning

CIs: Cyclic Imines

COP: Code of Practice

CTX: C-CTX and P-CTX: Ciguatoxins

DA: Domoic Acid

***Dinophysis* spp.:** Phytoplankton species associated with DSP

DSP: Diarrhetic Shellfish Poisoning (part of the lipophilic group)

DTXs: Dinophysistoxins

EFSA: European Food Safety Authority

EHS: Environmental Health Service, part of the Health Service Executive

epi-DA: Epi-domoic acid

Esters: Esters are naturally occurring derivatives of toxins which are also toxic

EURL: European Reference Laboratory

Food business operator: The natural or legal persons responsible for ensuring that the requirements of food law are met within the food business under their control. This includes dispatch centres and processing premises

FSAI: Food Safety Authority of Ireland

GYMS: Gymnodimine A, B, C, included in the cyclic imines group

HAB: Harmful Algal Blooms

HABS database: Harmful Algal Blooms Database (www.marine.ie/habs)

HACCP: Hazard Analysis and Critical Control Point

HBGV: Health-based guidance values

HPLC: High-performance liquid chromatography, a chemical analytical method

HSE: Health Services Executive

INAB: Irish National Accreditation Board

ISA: Irish Shellfish Association

ISO/IEC 17025:2005: International Standard of General Requirements for the Competence of Testing and Calibration Laboratories

iso-Das: Isodomoic acids A-H

LBM: Live Bivalve Molluscs. Filter-feeding shellfish with two shells. The legal requirements for LBM also relate to live echinoderms, live tunicates and live marine gastropods

LC-MS/MS: Liquid chromatography-quadrupole mass spectrometer, a chemical analytical method

Lipophilic toxins: This grouping is comprised of the following groups of toxins; okadaic acid group, esters of okadaic acid group toxins, pectenotoxins group, yessotoxins group and azaspiracid group

LOD: Limit of detection

LOQ: Limit of quantification

<LOD: Less than the limit of detection

<LOQ: Less than the limit of quantification

MI: Marine Institute

MSSC: Molluscan Shellfish Safety Committee

n.d.: Not Detected

NSP: Neurotoxic Shellfish Poisoning

OA: Okadaic Acid, a lipophilic toxin

PbTxs: Brevetoxins

Phytoplankton: Phytoplankton are microscopic plants that live in water

PlTxs: Palytoxins

PnTXs: Pinnatoxins, included in the cyclic imines group

Production area: A shellfish harvesting area defined by and classified by the Sea-Fisheries Protection Authority

Production period: The time period that a valid sample relates to during periods of harvesting. This is set by the sampling frequency and is normally a week or a month

***Pseudo-nitzschia*:** Phytoplankton species associated with ASP

PSP: Paralytic Shellfish Poisoning

PSTs: Paralytic shellfish toxins

PtTXs: Pteriatoxins A, B, C, included in the cyclic imines group

PTX: Pectenotoxins, included in the lipophilic toxin group

Sentinel sites: Production areas from around the coast that are sampled at a higher frequency and analysed for all toxins to give a representative view of toxicity

SFPA: Sea-Fisheries Protection Authority

SFPO: Sea-Fisheries Protection Officer

Shellfish coordinator: The SFPO with responsibility for overseeing the operation of the sampling in the Irish Shellfish Monitoring Programme

Shellfish harvesting area: Any sea, estuarine or lagoon area, containing either natural beds of bivalve molluscs or sites used for the cultivation of bivalve molluscs, and from which live bivalve molluscs are taken

Shellfish manager: The SFPO or Loughs Agency Officer with responsibility for a production area

Shellfish sampler: The industry representative or Loughs Agency Officer who carries out sampling in a production area

SPO: Senior Port Officer in the SFPA

Spp.: Species (plural)

SPXs: Spirolides, included in the cyclic imines group

STX: Saxitoxin

TTX: Tetrodotoxin

YTX: Yessotoxins, included in the lipophilic toxin group

CHAPTER 1. INTRODUCTION

Marine biotoxins are poisonous substances, which can accumulate in shellfish (and in certain fish), mainly as a result of feeding on plankton containing toxins. As a consequence, the shellfish industry must be supported by a robust monitoring programme so that consumers, both in Ireland and in other countries, can have confidence that the Irish shellfish they are purchasing is a safe product and that it meets the required legal health standards. The operation of the monitoring programme is carried out under the authority of the Sea-Fisheries Protection Authority (SFPA) as the competent authority for the enforcement of seafood safety legislation in Ireland, who, along with the Marine Institute (MI) as National Reference Laboratory operate a service level agreement for the Food Safety Authority of Ireland (FSAI) which has overall responsibility for food safety in Ireland.

Due to comprehensive food safety and environmental monitoring systems which meet European Union and international market requirements, Ireland has established an important share of the international aquaculture market.

Seafood is defined by Smith *et al*. (2010) as 'fish and shellfish harvested from capture fisheries and aquaculture production in marine and freshwater environments'. It is regarded as the most highly traded food type globally. According to Bord Iascaigh Mhara (BIM), (2014), the four main activities related to the seafood industry are: 1) Fishing (various fishing ports around the coast) 2) Fish farming (growing and farming finfish and shellfish) 3) Processing (companies and farmers generating high value produce) 4) Marketing (both domestic and international).

The seafood industry provides employment and produce for export, but it is vulnerable in two key aspects, it is highly dependent on functioning ecosystems (this may be threatened by for example, climate change) and is susceptible to disruption from human activities, e.g. over-fishing is detrimental, both of which threaten its sustainability.

The clean and unpolluted waters surrounding Ireland's 7,500 km of coastline make it a perfect environment for seafood. In 2012, more than 16,000 tonnes of shellfish were harvested in Ireland, valued at nearly €42.5 million. In 2013, the seafood industry's contribution to the Irish economy was valued at approximately €700 million, the shellfish industry generated €55.2 million of this by producing more than 24,000 tonnes of shellfish (BIM, 2013). In 2013, the shellfish industry employed 1,601 people directly; the entire seafood industry employs about 11,000 people (including fishermen, fish farmers, processors) predominantly in remote, rural coastal regions (BIM, 2014).

1.1 Aims and Objectives

Vigilance in ensuring the safety of Irish shellfish is of paramount importance. This report describes the risks posed by commonly encountered and novel or emerging toxins, including the:

1. Profile of harmful algae and their toxins in shellfish species and the geographical distribution of toxic events
2. Seasonality of toxins and their causative phytoplankton on a monthly basis over the annual cycle
3. Risk of non-regulated or novel toxins
4. Measures by which the toxins may be spread
5. Monitoring regimes for harmful algal blooms and for toxins in shellfish currently in place aimed at controlling these risks
6. Results for the monitoring programmes for the period 2011 to 2013
7. A regulatory maximum level exposure assessment scenario for Irish consumers of shellfish provided
9. Recommendations relating to research, regulation and enhanced monitoring

CHAPTER 2. TOXINS OF CONCERN TO IRISH CONSUMERS

Marine biotoxins are a wide range of naturally occurring substances with varying solubility and toxicokinetics produced by microscopic planktonic algal cells. They are often grouped into structurally similar toxin families. Bivalve shellfish feed on these algal cells via filtration and can accumulate a variety of toxins. If sufficiently high levels of toxin are ingested and accumulated by the shellfish, consumers who eat the shellfish may become sick. In general, the rate of shellfish elimination of toxins, and as a consequence, the potential to cause harm to consumers depends on the filtration and clearance rates of the bivalve. The following contributing factors to toxin accumulation by shellfish have been identified:

- Species dependent body weight (Bricelj and Shumway, 1998), with increasing body weight leading to decreased filtration and clearance rates (Gosling, 2003)
- Water temperature, with lower temperatures resulting in slower toxin loss due to decreased filtration and clearance rates (Gosling, 2003)
- Water salinity, with shellfish being exposed to salinities they are unused to, decreasing their filtration and clearance rates (Gosling, 2003)
- Location of toxin storage within the shellfish whereby toxins usually accumulate in the digestive tract and the hepatopancreas can act as a store
- Shellfish feeding rates whereby as food availability increases, filtration and clearance rates similarly rise to a certain point but will then decrease as food levels continue to increase (Strohmeier *et al.*, 2009)

There is currently no functional way to prevent uptake of toxin by shellfish or to remove the toxin after harvesting. Extensive monitoring of the marine environment and produce (especially when algal blooms may occur), regular inspection of seawater surrounding facilities for the presence of toxin-producing species of phytoplankton and cysts of dinoflagellates, and adherence to species-specific regulations are essential. Closure of production areas, if the regulatory limits have been exceeded, is necessary. The main biotoxin groups monitored may be categorised as lipophilic, hydrophilic and novel/emerging toxins.

2.1 Lipophilic Toxins

Lipophilic marine biotoxins are an assorted range of naturally occurring toxins found in marine phytoplankton (such as *Dinophysis*) and the shellfish that consume them. These toxins are often grouped into 'Azaspiracid Poison (ASP)', 'Diarrhoeic Shellfish Poisons (DSP)' and 'Yessotoxins (YTX)' groups. These toxin producing phytoplankton, taken up as part of shellfishes' diet, can accumulate in the fat rich tissues. Symptoms of lipophilic toxins are mainly reported as being gastrointestinal and include nausea, vomiting, diarrhoea and stomach cramps (Munday and Reeve, 2013). Clinical signs appear acutely and may last for two to four days. The symptoms of the DSP toxin group and azaspiracid shellfish poisoning (AZP) group are diarrhoea, nausea, vomiting and abdominal pain (gastrointestinal upset). Pectenotoxins which are included in the DSP group are not believed to result in human illness. There is also no evidence that the YTX toxins have resulted in human toxicity (Toyofuku, 2006; Munday and Reeve, 2013).

2.1.1 Azaspiracid poisoning

Azaspiracids (AZAs) are a group of non-neurotoxic lipophilic toxins produced by a dinoflagellate. AZAs are found in many species of filter-feeding bivalve molluscs, but they are found most commonly in mussels in Ireland (EFSA, 2008).

The causative organisms are *Azadinium spinosum* and *Amphidoma* species, which result in the production of AZA toxin and more than 30 analogues. When taken in by molluscs, AZA analogues undergo metabolism and further derivatives are formed, e.g. AZA-3 is formed when AZA-1 is demethylated, AZA-4, -5 are formed when AZA-3 is

hydroxylated. AZP and its analogues are not concentrated solely in the hepatopancreas of shellfish, but throughout its tissues. AZAs found in shellfish are not broken down at cooking temperatures (EFSA, 2008).

The regulatory limit for AZAs in bivalve molluscs destined for human consumption is 0.16 µg/g for AZA-1, AZA-2 and AZA-3, expressed as AZA equivalents (Regulation (EC) No 853/2004). AZA 1-3 are considered the most toxicologically significant; AZA 4-33 are not legislated for and present an unknown public health risk.

There are a number of emerging issues associated with AZA in shellfish that may require further investigation, for instance, EU regulation stipulates that only raw shellfish are tested, yet shellfish are often cooked prior to consumption. Analysis of raw and heat-treated mussels (*Mytilus edulis*), naturally contaminated with AZAs, reveals significant differences in toxin profiles due to heat induced chemical conversions. Recent studies have shown high levels of AZA-3 and AZA-6 in some samples that were otherwise below the analytical limit of quantification before heating (McCarron *et al*. 2009; Kilcoyne *et al*. (2015).

2.1.2 Diarrhoeic shellfish poisoning

The toxins okadaic acid (OA), dinophysistoxins (DTXs) and pectenotoxins (PTXs) which may cause DSP are heat stable and found in some species of shellfish, predominantly those that are filter feeding bivalve molluscs, and cause gastrointestinal upset. Dinoflagellates that produce the toxin, have a global distribution.

The causative organisms are *Dinophysis* species and *Prorocentrum* species, resulting in the production of OA, DTXs and PTXs. *Dinophysis* and *Prorocentrum* species produce OA and DTXs; PTX is produced only by *Dinophysis* species.

The regulatory limit for DSP toxins (OA, DTXs and PTXs combined) is 0.16 µg/g expressed as OA equivalents (Regulation (EC) No 853/2004). The risks associated with DSP are not fully known as not all derivatives of OA are analysed. Exposure to DSP toxins may increase risk of cancer, however mutagenic and genotoxic studies to date are conflicting, and therefore, further investigation is warranted (Fujiki, 1988, Suganuma, 1988, Dörr, 2014). OA can cross the placenta however, the effect on the foetus *in-utero* is unknown and teratogenic studies would be beneficial. A chronic toxicity study would be useful to assess the potential of OA to accumulate in body tissues.

2.1.3 Yessotoxins

YTXs have been identified in filter feeding bivalve molluscs in various locations around the world.

The causative organisms are *Protoceratium reticulatum* (primarily), *Patinopecten yessoensis*, *Lingulodinium polyedrum* and *Gonyaulax spinifera*. The regulatory limit for YTXs (Regulation (EC) No 853/2004) is 3.75 µg/g expressed as YTX equivalents.

2.2 Hydrophilic Toxins

Hydrophilic marine biotoxins, which include paralytic shellfish poison (PSP) and amnesic shellfish poison (ASP) toxins, are an assorted range of naturally occurring toxins found in marine phytoplankton (such as *Alexandrium* spp. and *Pseudo-nitzschia* spp.) and the shellfish that consume them. Hydrophilic toxins, taken up as part of shellfishes' diet, are less persistent than the fat soluble toxins and are often expelled soon after contamination without the long lasting intoxication often seen in lipophilic toxins.

2.2.1 Amnesic shellfish poisoning

ASP occurs following the ingestion of contaminated shellfish that have accumulated sufficient levels of ASP toxin domoic acid (DA) and its isomers epi-domoic acid (epi-DA) and isodomoic acids A-H (iso-Das). These have been identified in North America and some European countries and can result in serious gastrointestinal and/or neurological symptoms after consumption. DA can be transformed into epi-DA during long-term storage; ultra-violet light exposure can result in transformation to epi-DA and iso-Das (EFSA, 2009).

The causative organisms are *Pseudo-nitzschia* species. DA is heat stable and thus not destroyed by cooking but its concentration is reduced by boiling and steaming due to partial leaching of DA into the cooking liquid, as it is hydrophilic (EFSA, 2009).

The clinical signs of DA may include gastrointestinal symptoms, e.g. abdominal cramps, vomiting, and diarrhoea, and neurological symptoms, e.g. disorientation, confusion, seizures, permanent short term memory loss and coma, and can prove fatal (Munday and Reeve, 2013).

Regulation (EC) No 853/2004 requires that bivalve molluscs for human consumption do not exceed 20 µg/g of DA.

The risks associated with ASP are not fully known as there has been an emphasis on toxicological studies for DA, but not for its isomers or stereoisomers, for which there is a lack of epidemiological data and hazard characterisation.

2.2.2 Paralytic shellfish poisoning toxins

Saxitoxin (STX) and its analogue paralytic shellfish toxins (PSTs) are potent neurotoxins found in specific marine algae species, for example *Alexandrium*. More than 30 different STX analogues have been identified (EFSA, 2009b). Many *Alexandrium* species are found globally (Taylor *et al.*, 1995).

A known trait of *Alexandrium* blooms is that they regularly occur in the same location annually (Giacobbe *et al.*, 2007) due to the cysts they produce which can remain dormant in marine sediments for years. According to Cosgrove *et al.* (2014) a 'sheltered channel, bay or estuary' can be the site of long-term infestation by *Alexandrium* as they are the perfect habitats for propagation. The specific local hydrography in Cork Harbour predisposes to PSP outbreaks (Cosgrove *et al.*, 2014) due to the lagoon environment and gyre locations in particular. This is the 'retentive environment' that *Alexandrium* species cysts require.

Significantly, Cosgrove *et al.*, (2014) concluded that the intensity of the *Alexandrium* species bloom is independent of the cyst density recorded during the previous winter, i.e. intense blooms, which are recorded every 7-8 years, are not sufficient to allow *Alexandrium* species to persist long-term; lower intensity blooms provide enough cysts to "ensure the annual inoculation of the water column".

The causative organisms are *Alexandrium spp*. *Pyrodinium bahamense* and *Gymnodinium catenatum*. The toxicity of these dinoflagellates is due to a mixture of STX derivatives and the composition of the mixture differs depending on the species producing it and/or the region of occurrence. The mechanism of action is similar to tetrodotoxin (TTX) – it blocks the voltage gated sodium channel, which dramatically slows or stops the propagation of the action potential and results in progressively decreasing muscular function.

Symptoms range from tingling/numbness around the lips, nausea, vomiting, diarrhoea, to increasing muscular paralysis and respiratory difficulty and can prove fatal (Munday and Reeve, 2013). Variations have been observed in human sensitivity to PSP toxins, which may be due to differing proportions of various PSP toxins in the shellfish.

2.3 Emerging/Novel Toxins

This group of both lipophilic and hydrophilic unregulated toxins not seen routinely in Irish shellfish samples are potentially a threat by spreading of the causative organisms. This spreading may be facilitated through changing environmental conditions or through actual physical movement of, for instance, shellfish or ballast water discharge.

2.3.1 Neurotoxic shellfish poisoning

Neurotoxic shellfish poisoning (NSP) is caused by the consumption of shellfish contaminated by brevetoxins (PbTxs) and its analogues. This group of more than ten natural toxins are produced by dinoflagellates and can result in serious gastrointestinal and neurological symptoms in consumers. NSP outbreaks in humans are relatively rare due to effective monitoring programmes. The causative organism (a planktonic dinoflagellate *Karenia brevis*) is known in the Gulf of Mexico, Carribean and New Zealand coastal regions, (Watkins *et al*, 2008), but has not to date been detected in Irish (or adjacent) coastal waters.

NSP toxins can result in symptoms ranging from nausea, vomiting and diarrhoea, to paresthesia, ataxia and disorientation (Munday and Reeve, 2013). There is a lack of data with regard to PbTxs and there is currently no regulatory limit in Europe. Studies are required in order to establish a suitable regulatory limit. A limit of 800 µg/kg in shellfish has been set in Australia, New Zealand and the United States.

NSP poses a potential risk due to the lack of a regulatory limit for the associated toxins and subsequent lack of monitoring in Europe. As exposure may be via ingestion of contaminated seafood or inhalation of aerosolised toxin, vigilant monitoring is needed to ensure the phytoplankton remains absent in Irish waters.

2.3.2 Ciguatera fish poisoning

Ciguatoxins (CTX: C-CTX and P-CTX) are lipid soluble and can accumulate up through the food chain and many fish (Swordfish, Great barracuda, Horse-eye Jack etc.) and other marine organisms, e.g. *Turbo argyrostoma* – a marine snail, are found with CTX.

The causative organism is a dinoflagellate – *Gambierdiscus toxicus*, found in tropical waters. CFP can produce symptoms such as vomiting, diarrhoea and tingling (Munday and Reeve, 2013).

CFP is currently only endemic in tropical and subtropical areas; however it may be imported in fresh or frozen fish and hence poses a risk. Therefore, regular chemical analysis of imported fish from endemic regions to Ireland for CTXs is prudent as CFP is the most common non-bacterial cause of foodborne illness associated with ingestion of fish (Friedman *et al.*, 2008).

A further risk factor is potential climate change, which may alter the typical habitat in which CFP is found.

2.3.3 Cyclic imines

This group encompasses a large and varied range of causative organisms and their corresponding toxins (Table 1).

Table 1. Examples of cyclic imines

Toxin	Causative organism	Occurrence
Gymnodimine A, B, C (GYMS)	*Karenia selliformis*	Identified in shellfish imported into Europe but not in shellfish produced in Europe (EFSA, 2010)
Spirolides (SPXs)	Causative organisms: *Alexandrium ostenfeldii*, *A. peruvianum*	Identified in shellfish in a number of European countries (EFSA, 2010)
Pinnatoxins (PnTXs)	*Vulcanodinium rugosum*	Identified in Norwegian shellfish (EFSA, 2010)
Prorocentrolide A	*Prorocentrum lima*	
Prorocentrolide B	*Prorocentrum maculosum*	
Pteriatoxins A, B, C (PtTXs)	Isolated from *Pteria penguin* but causative organism not yet identified. Studies suggest PtTXs are converted from PnTXs in shellfish (EFSA, 2010)	Not identified in shellfish in Europe (EFSA, 2010)
Spiro-prorocentrimine	Unidentified benthic *Prorocentrum* sp.	
Symbioimine	*Symbiodinium* sp.	

According to Munday and Reeve (2013), no human illness has to date been associated with cyclic imines (CIs).

CIs are not currently regulated, as to date, no association has been found between CIs and human illness. However, continuous data collection and further studies, particularly on chronic exposure, should be undertaken to establish whether these toxins represent a risk to human health.

2.3.4 Palytoxins

Palytoxins (PlTxs) are water soluble and associated with dinoflagellates. The causative organisms are *Palythoa* spp., *Ostreopsis* spp, both which produce palytoxin and palytoxin derivatives.

The symptoms associated with palytoxins include muscle pain, myoglobinuria, respiratory difficulties and cyanosis and may result in death due to respiratory arrest (Munday and Reeve, 2013).

The clinical signs of palytoxin ingestion are severe, however thus far no health-based guidance value (HBGV), e.g. Tolerable Daily Intake (TDI) or Acute Reference Dose (ARfD), has been set and research to develop such values is a priority (Botana, Luis M, 2014). Consequently, there is currently no regulatory limit in place.

The risk to Irish consumers could increase if *Ostreopsis* spp. spread from their current habitat range which is limited in northern extent to the warmer waters of the Mediterranean. Therefore, data collection and establishment of a regulatory limit are essential.

2.3.5 Tetrodotoxins

Tetrodotoxins (TTXs) are potent neurotoxins and considered the 'most lethal toxins in the marine environment' (Botana, 2014). TTXs are the most common cause of fatal marine poisoning globally (Isbister and Kiernan, 2005).

The toxins have been isolated from a wide variety of marine organisms, but are most commonly associated with pufferfish.

Similar to STX, TTXs block sodium channels which leads to numbness of face and extremities, respiratory paralysis and death (Munday and Reeve, 2013).

Although poisonous fish from the family *Tetraodonitae* (from which TTXs originate) cannot be sold in the EU (Regulation EC 853/2004 and Regulation EC 854/2004), this does not rule out accidental exposure.

While not known to Irish waters, there is a risk of importing TTXs in marine produce. Recently there has also been detection of TTXs in European bivalve shellfish and gastropods. This was detected firstly in a non-fatal human intoxication following consumption of the contaminated sea snail *Charonia lampas lampas* (a gastropod) harvested in Spain (Rodriguez, 2008). It was also shown that TTXs are present within the temperate waters of the United Kingdom, along the English Channel, and can accumulate in filter-feeding molluscs (Turner *et al.*, 2015).

CHAPTER 3. THE SPREAD OF EMERGING TOXINS

A non-indigenous species can spread in two main ways – jump dispersal or range expansion (van den Bergh *et al.*, 2002). Jump dispersal involves transport via ballast water or with exotic samples for aquaculture practices. These are referred to as 'introduced species'. Range expansion occurs when temperature changes allow species from warmer regions to migrate and, therefore, are not considered as introduced species. Whereas the risk of jump dispersal can be reduced, the risk of range expansion cannot.

The main mechanisms for spread of emerging toxins comprise:

- Ballast water (jump dispersal)
- Climate change (range expansion)
- Ocean acidification
- Import/export of shellfish produce (jump dispersal)

In all scenarios, the temperature of the region will dictate if the exotic organism will survive throughout the year, and if it can reproduce (van den Bergh *et al.*, 2002).

3.1 Ballast Water

Ballast water is a ship's safety mechanism. It provides stability at sea but unfortunately it may also create a serious environmental issue. Phytoplankton are abundant in ships' ballast water as they move with the water column they live in and can thus be transported internationally (McCarthy and Crowder, 2000). Ballast water is the most common way in which micro-algae are shipped to a non-indigenous region (van den Bergh *et al.*, 2002).

Introduction of new species to an area can result in:

- Production of toxic blooms (Hallegraeff *et al.*, 1988)
- Development of brown tides which damage eelgrass beds (Cosper *et al.*, 1987)
- Blockage of fishing nets with bloom mucilage (Boalch and Harbour, 1977)

This poses a risk to food production and safety, aquatic food chain stability and human health (Shumway and Cembella, 1993; Scholin *et al.*, 2000; Carmichael, 2001).

Ballast water has been implicated in the introduction of many phytoplankton species into new regions for decades, e.g. the movement of *Gymnodinium catenatum* into Spanish and Australian waters from Japan. The introduction of *G. catenatum* could result in long-term negative effects, from damage to fisheries and the marine ecosystem to the risk to human health. In 2004, the International Maritime Organisation (IMO) adopted a major initiative; the International Convention for the Control and Management of Ships' Ballast Water and Sediments. This convention requires all vessels to employ a Ballast Water Management Plan.

McCollin *et al.* (2007) examined the efficacy of ballast water exchange at decreasing phytoplankton variety and abundance and concluded that there was an overall reduction but it was not consistent. The authors suggested that water depth during the exchange, the season and the method of exchange, were influential.

Filtration has been investigated as a method of reducing the introduction of non-native species of phytoplankton. Cangelosi *et al.* (2007) found that 25 or 50 µm size filters may be a useful means to minimise the quantity of organisms released by ships but an additional treatment step would be required to further reduce the risk and meet IMO's discharge standards. Gregg *et al.* (2007) investigated the efficacy of three different ballast water biocides (SeaKleen®, Peraclean Ocean®, Vibrex®) on dinoflagellate cysts, bacteria and microalgae. They found that low water temperature, differing light conditions and the presence of humus-rich seawater and ballast water sediment reduced the performance of the biocides. The authors found that cost effectiveness was poor and there was a corrosive effect on the ship.

3.2 Climate Change

The expansion in range of harmful algae blooms (HABs) species (or indeed the retraction of their existing distribution) is one of the changes likely to occur in response to climate change. At a recent conference in Sweden (Joint PICES-GEOHAB-ICES Symposium, 2015) on HABs and climate change, it was agreed that "*climate change, including warmer temperatures, changes in wind patterns, ocean acidification and other factors will influence harmful algal blooms*", but it was also concluded that data are lacking to support these hypotheses. The apparent emergence of *Gambierdiscus toxicus* and associated ciguatera fish poisoning in the Canary Islands suggest that this could be an important sentinel site to study expansion and increased occurrence of this HAB at a site that did not experience problems in the past. However, *G. toxicus* will have to be sampled differently than standard pelagic phytoplankton species. This organism is an example of a key species for understanding the impact of climate on wild fisheries.

An indirect impact involves how the effect of human population expansion and land use affects the nutrient input and balance in marine ecosystems. Climate change is creating additional rainfall in countries such as the UK and Ireland and this in turn is increasing the risk of run-off which could lead to an imbalance in the Nitrogen:Phosphorus ratio in the phytoplankton environment (Callaway, *et al.*, 2012). Nitrogen has been shown to be necessary for the synthesis of STX and an increase could lead to increased production of this toxin (Touzet *et al.*, 2007). The increase of sea surface and seasonal temperatures could see an increase in the growth rate and occurrence of HAB events as these involve flagellates, which, through their cellular lifecycle, favour increased temperatures and increased stability in the water column (Peperzak, 2003; Bresnan *et al.*, 2013).

3.3 Ocean Acidification

As the concentration of CO_2 in the atmosphere increases, a proportion of the extra CO_2 is moved into the ocean, which leads to a multitude of changes to the seawater and its inhabitants (Doney *et al.*, 2009). There is concern as to how marine ecosystems will respond to these alterations. As a result of increased atmospheric CO_2 levels, surface sea water has become more acidic with a decrease in pH of 0.1 units being recorded since 1750 (Frost *et al.*, 2012). This phenomenon is not expected to affect the oceans around Ireland in the short-term but could have long-term effects (Callaway *et al.*, 2012).

Main biological consequences include:

- Changes in the acid/base balance and trace metal levels may lead to alterations in phytoplankton growth
- Changes in pH levels may influence various physiological processes, e.g. photosynthesis, respiratory metabolism and transport of nutrients
- Increased solubility of calcium carbonate minerals, which are used by many organisms to build skeletons and shells, may result in a decrease in the overall calcification. Decreased calcification could have a major impact on shellfish species

Ocean acidification is a global issue which will affect many regions and marine ecosystems, from coral reefs to the deep sea.

3.4 Import/Export of Shellfish Produce

The shipment and transport of shellfish from point of origin to market provides an opportunity for movement of biotoxins to non-indigenous regions, i.e. jump dispersal. Live shellfish containing cysts pose a huge risk of importing toxins. In order to prevent introducing toxic micro-algae into Northern European waters (the North Sea), in 1987 the importation of bivalve shellfish from regions other than the German, Dutch or Danish Wadden Sea was prohibited (van den Bergh *et al.*, 2002). However, with the establishment and further development of the European Union, trade has become more open and it is harder to monitor movement of live shellfish (van den Bergh *et al.*, 2002).

CHAPTER 4. SHELLFISH SAFETY MONITORING PROGRAMME

The Irish Sampling Plan for Shellfish Biotoxin Monitoring and Phytoplankton Monitoring is published by the FSAI (FSAI, 2014). This Code of Practice for the Irish Shellfish Monitoring Programme was developed by the Molluscan Shellfish Safety Committee (MSSC) through consultation with all stakeholders. It outlines how Ireland meets its obligations to protect consumers and comply with the requirements laid down in Irish and European legislation.

The monitoring scheme is presented in this document and is a risk-based approach to ensure that all shellfish production areas are tested to account for the shellfish species being produced, the shellfish toxins that may be encountered and, at an appropriate frequency, to detect these toxins before they present any risk to human consumers. The monitoring of shellfish toxins is based on the best available scientific evidence and is undertaken in an independent, objective and transparent manner. The management of risk relating to shellfish toxins takes into account, the results of ongoing shellfish and phytoplankton monitoring and on occasion, may be supplemented by opinions of the MSSC, as well as other factors that may be relevant to the matter under consideration.

The programme monitors all commercial shellfish production areas in Ireland for the presence of regulated toxins, as specified under Regulation (EC) 853/2004. Shellfish production areas are classified by the DAFM under Statutory Instrument No.147 of 1996 which implements the EC Council Directive 91/492/EEC on laying down the health conditions for the production and placing on the market of live bivalve molluscs.

In accordance with Annex III, Section VII, Chapter V of Regulation (EC) 853/2004, the following shellfish toxins are required to be measured to ensure that the thresholds indicated are not exceeded.

Table 2. Legislative maximum limits laid down for biotoxins

Toxin Group (Lipophilic or Hydrophilic)	Toxins	Regulatory Limit	Reported As
DSP/Okadaic acid group (Lipo)	OA, DTX1, DTX2, including their esters	160 µg/kg	**OA equivalents (calculated as toxicity equivalency factors of each toxin to OA)**
AZP/Azaspiracids group (Lipo)	AZA1, AZA2 and AZA3	160 µg/kg	**AZA-1equivalents (calculated as toxicity equivalency factors of each toxin to AZA-1)**
PTX/Pectenotoxins group (Lipo)	PTX1 and PTX2	160 µg/kg	**Sum of PTXs**
YTX/Yessotoxins group (Lipo)	YTX, 45 OH YTX, homo YTX and 45 OH homo YTX	3.75 mg/kg	**YTX equivalents (calculated as toxicity equivalency factors of each toxin to YTX)**
PSP/Saxitoxin group (Hydro)	dcGTX23, dcSTX, GTX2,3, GTX5, STX, C1,2, GTX1,4, NEO, dcNEO	800 µg/kg	**STX diHCl equivalents (calculated as toxicity equivalency factors of each toxin to STX)**
ASP/Domoic Acid group (Hydro)	DA and epi-DA	20 mg/kg	**Sum of domoic acid and epi-domoic acid**

Chapter II (Part B) of Annex II to Regulation (EC) 854/2004 specifies the monitoring requirements for classified relaying and production areas for live bivalve molluscs and, by analogy, to live echinoderms, live tunicates and live marine gastropods.

The Shellfish Safety Monitoring Programme, as carried out in Ireland, has two main strands:

a. Phytoplankton count and monitoring

b. Analysis of produce

4.1 Phytoplankton Identification, Count and Toxin Analysis

Phytoplankton are primary producers, consequently many marine food chains rely on them as a food source, which makes them drivers of the marine ecosystem.

The importance of phytoplankton cannot be overstated, according to Boyce *et al*. (2010), they account for 'approximately half the production of organic matter on Earth'. Marine phytoplankton can influence the volume and variety of marine organisms, assist in the functioning of the marine ecosystems and set an upper limit on fishery yields.

MI carries out a Phytoplankton Monitoring Programme which functions as an early indicator of potential toxins that may be present and acts as a trigger for monitoring of bivalve molluscs in production regions. According to Regulation (EC) No 854/2004, intermittent monitoring to check for the specific toxin-producing phytoplankton must be carried out in production areas, as described in the next paragraphs.

Sampling plans to check for the presence of toxin-producing plankton in shellfish production areas and waters where shellfish are relayed for on-growing and for biotoxins in live bivalve molluscs, must take particular account of possible variations in the presence of plankton containing marine biotoxins. Sampling must comprise:

1. Periodic sampling to detect changes in the composition of plankton containing toxins and their geographical distribution. Results suggesting an accumulation of toxins in mollusc flesh must be followed by intensive sampling
2. Periodic toxicity tests must be undertaken using those molluscs from the affected area most susceptible to contamination

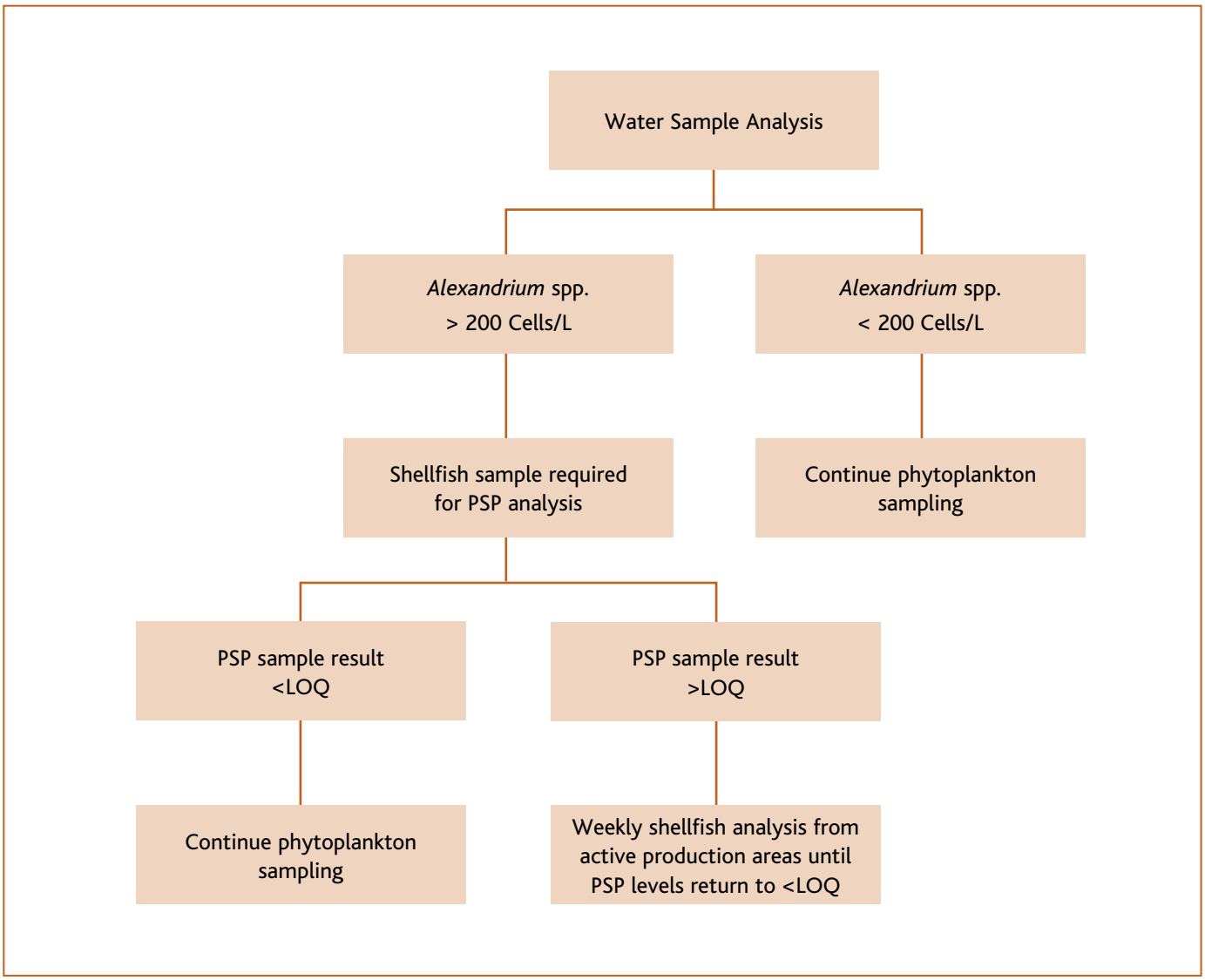
The sampling frequency for toxin analysis in the molluscs is, as a general rule, to be carried out weekly during the periods at which harvesting is allowed. This frequency may be reduced in specific areas, or for specific types of molluscs, if a risk assessment on toxins or phytoplankton occurrence suggests a very low risk of toxic episodes. Equally, it is to be increased where such an assessment suggests that weekly sampling would not be sufficient.

The risk assessment is to be periodically reviewed in order to assess the risk of toxins occurring in the live bivalve molluscs from these areas.

Phytoplankton monitoring requires a sampling point that is located in an area that will be of a predictive nature (usually close to the incoming water currents). Selection of the water column is very important and is helped by knowledge of the region being sampled. Selection of the species to be monitored is necessary and also depends on knowledge of the region. A rapid increase in specific (toxin-producing) phytoplankton suggests the need for further sampling to identify if there are potential harmful levels of toxins in shellfish. Figure 1 provides an example of phytoplankton monitoring and consequent toxin testing.

Figure 1. The decision tree for the monitoring of *Alexandrium* spp. and PSP in production areas

(outside of Cork Harbour)
(Source: Code of Practice for the Irish Shellfish Monitoring Programme, 2013)



LOQ – Limit of quantification of the analytical method

4.2 Analysis of Produce

MI and the SFPA oversee the analysis of produce as part of the service contract agreed with the FSAI. Figure 2 outlines the parameters and regulations that must be met and Table 3 provides an overview of the methods of analysis used for each toxin group. Under the supervision of the SFPO, fishermen collect samples of farmed and wild shellfish from representative and fixed points in the harvesting regions (production areas) and submit them to the MI for analysis. The production areas and sampling points for shellfish produce are shown in Figure 3.

Figure 2. Legal regulations for toxins and detection methods

(Source: Botana, 2014)

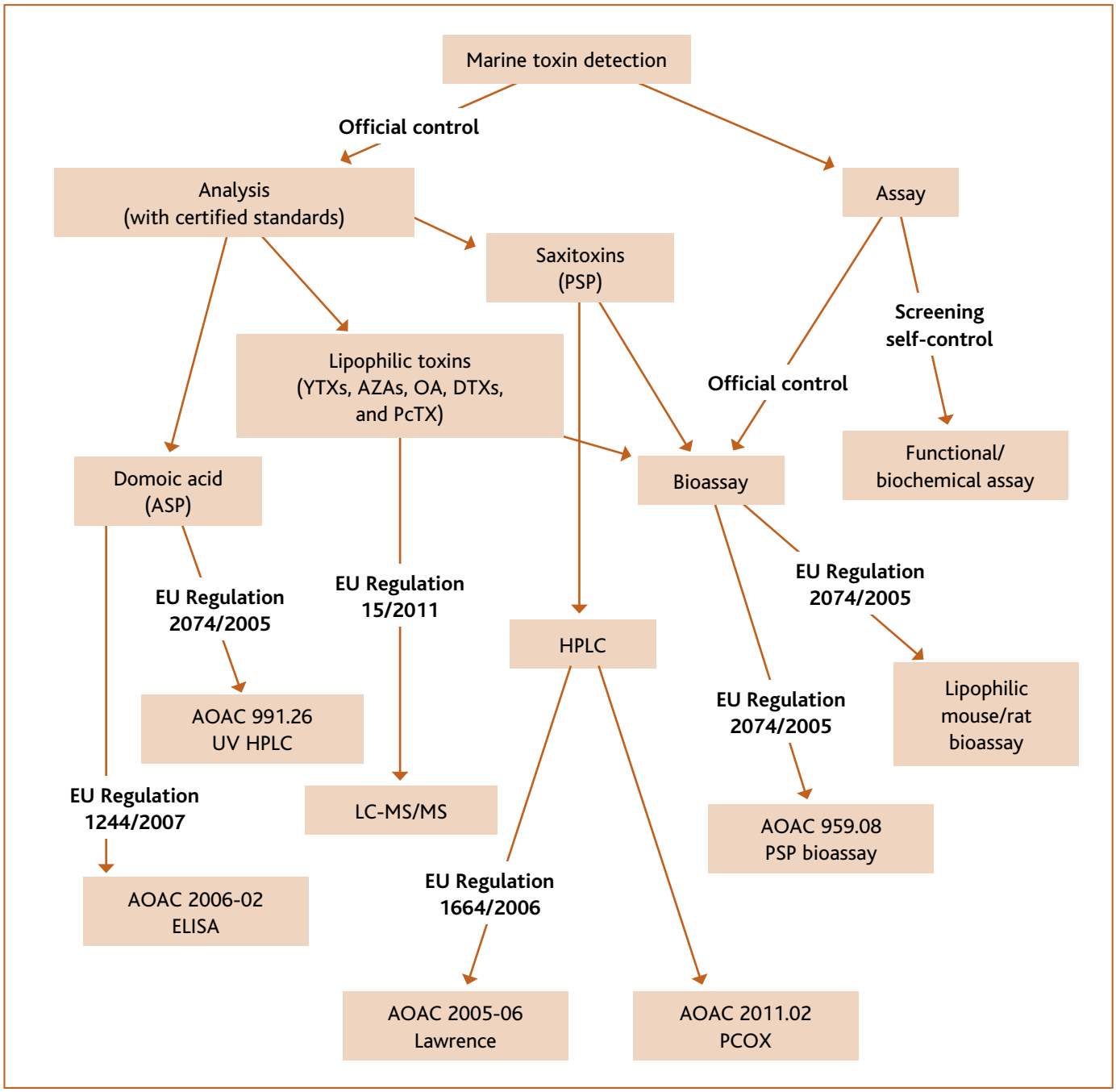


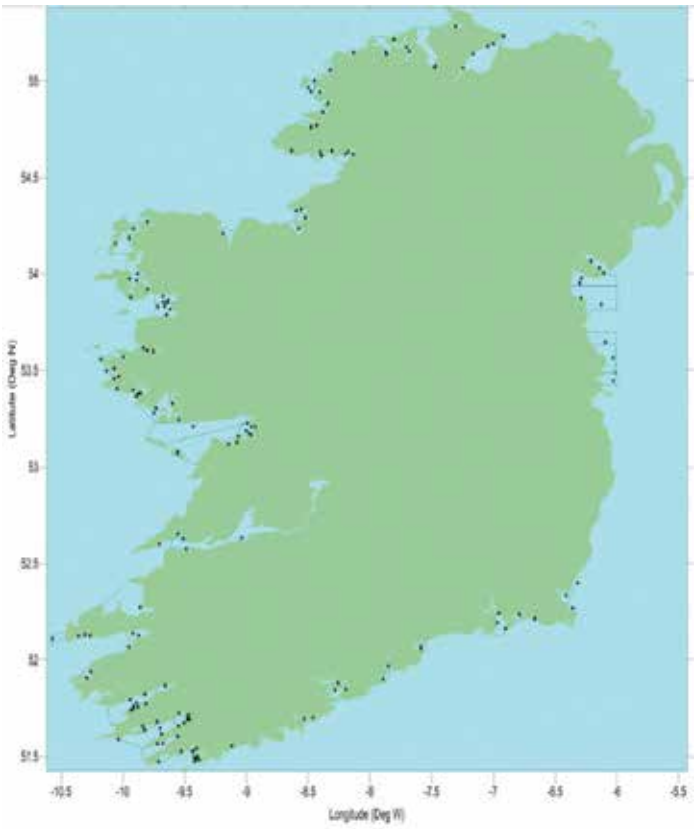
Table 3. Biotoxin methods of analysis

(Source: Code of Practice for the Irish Shellfish Monitoring Programme, 2013)

Toxin Group (Lipophilic or Hydrophilic)	Toxins	Method of Analysis
Method of Analysis	OA, DTX1, DTX2, including their esters	LC-MS/MS EURL- LCMSMS
AZP/Azaspiracids group (Lipo)	AZA1, AZA2 and AZA3	LC-MS/MS EURL- LCMSMS
PTX/Pectenotoxins group (Lipo)	PTX1 and PTX2	EURL- LCMSMS
YTX/Yessotoxins group (Lipo)	YTX, 45 OH YTX, homo YTX and 45 OH homo YTX	LC-MS/MS EURL- LCMSMS
PSP/Saxitoxin group (Hydro)	dcGTX23, dcSTX, GTX2,3, GTX5, STX, C1,2, GTX1,4, NEO, dcNEO	HPLC FD Lawrence Method AOAC 2005/06
ASP/Domoic acid group (Hydro)	DA and epi-DA	AOAC 2006/02 HPLC UV (also in-house LCMSMS Screening method)

Figure 3. Map of production areas and sampling points for shellfish species in the Republic of Ireland

The lines indicate production areas; the circles indicate location of sampling sites within the production areas



To minimise risk and prevent toxins entering the food chain, each harvesting region is allocated a biotoxin status based on the test results for that region (FSAI, 2013). The status assigned to a region is based on the last sample supplied (see Table 4). Two samples taken 48 hours apart are required to re-open a region that has been closed or is dormant. Only shellfish from areas with the status 'open' can be harvested and placed on the market. Irish and European Law requires that food business operators (producers, manufacturers, distributors, retailers, caterers) be held responsible for the safety of any food they choose to place on the market. Producers (farmers) must ensure that harvesting only takes place when production regions are deemed safe.

Table 4. Production area status for the lipophilic toxin group and PSP

(Source: Code of Practice for the Irish Shellfish Monitoring Programme, 2013)

Status	Explanation
Open	The most recent valid sample is below the regulatory limit. The production area is open for harvesting for that species until the end of the production period.
Closed	The most recent valid sample has exceeded the regulatory limit or the open status has lapsed. The production area is closed for the harvesting or lifting of shellfish unless the express permission of the SFPA has been obtained for the movement of shellfish.
Closed Pending	The most recent valid sample is below the regulatory limit but there is no previous valid sample. The production area is closed for harvesting for that shellfish species until a second result below the limit is obtained.

According to the Code of Practice for the Irish Shellfish Monitoring Programme (Biotoxins), the aim is to guarantee that '*Irish live bivalve molluscs placed on the market meet the highest standards of food safety and so maintain the excellent reputation of Irish shellfish*'.

In production areas where more than one shellfish species is produced, and in the same manner as for phytoplankton monitoring, an '*indicator species*' may be selected for regular testing while the other commercial species may be tested less frequently. Once the indicator species goes toxic, the frequency of testing of other shellfish species is increased to allow for species-specific openings and closures within the production area. An indicator species is selected based on it accumulating a toxin before other species do, e.g. mussels on long-lines allow prediction of potential toxicity in neighbouring oysters. Indicator species are a "*sensitive indicator of a chemical contaminant, biological toxin or pathogen, due to their ability to concentrate or integrate exposures within a food web ecosystem*" (Schwacke, 2013).

In practice, the MI reviews test results from the phytoplankton and shellfish analysis combined with other data, e.g. seasonal changes, to perform the risk assessment. This is then used to identify if the sampling interval should be increased or decreased for shellfish species in specific production regions. Permissible sampling frequencies and gaps are shown in Figures 4 and 5. Decisions on the status of production areas are made based on the most up-to-date results and this determines whether the area is allowed to be harvested or not (Figure 6).

The risk status of production regions are kept under review and are updated as required. When the MI concludes that changes to the sampling frequency are required, the SFPA shellfish co-ordinator and subsequently, sample managers and samplers, are informed. The laboratory analysis reports issued by the MI will also reflect this change. Changes to the sampling interval and its application to a production area usually takes at least two weeks to allow for essential arrangements to be made and to facilitate a smooth transition.

Figure 4. Permissible sampling frequencies

Sampling frequency required for samples to be valid

For a sample to be valid it must be taken at least 48 hours after any previous valid sample.

The **maximum gap** allowed between valid samples will depend on the sampling frequency in force:

- When the sampling frequency is **weekly**, a sample should be submitted each week, with no more than **12 days** between sample dates. The sampling week starts on a Sunday and ends the following Saturday
- When the sampling frequency is **fortnightly**, a sample should be submitted each fortnight, with no more than **19 days** between sample dates. The sampling week starts on a Sunday and ends the following Saturday week
- When the sampling frequency is **monthly**, a sample should be submitted each calendar month, with no more than **38 days** between sample dates

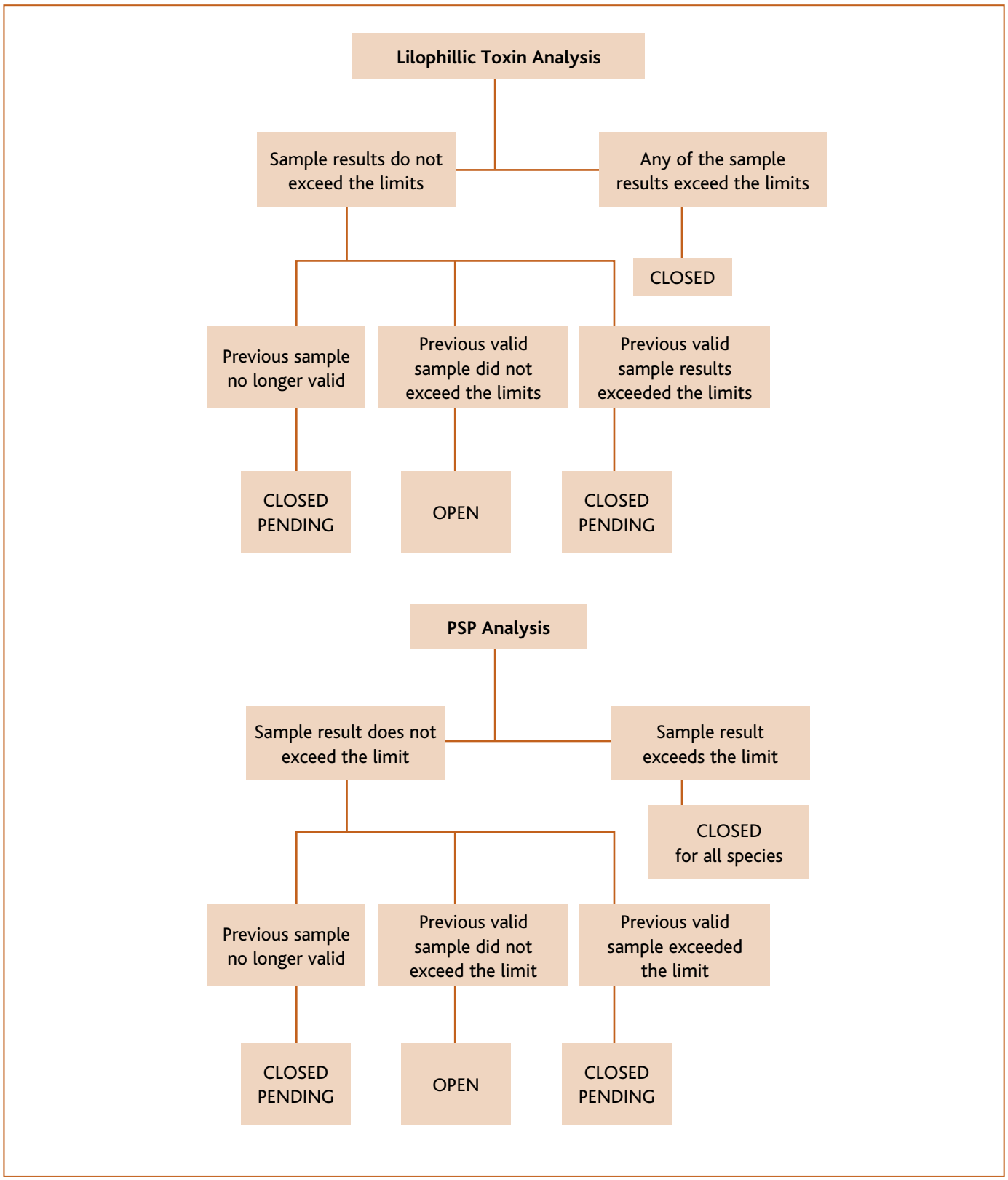
If the period of validity of a sample has finished and no new valid sample has been taken, the production area defaults to a closed status.

Figure 5. Permissible sample gaps when on weekly, fortnightly or monthly sampling frequency

Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

Figure 6. Lipophilic and PSP decision tree

(Source: Code of Practice for the Irish Shellfish Monitoring Programme, 2013)



CHAPTER 5. MONITORING AND OCCURRENCE OF MARINE BIOTOXINS IN IRELAND, 2011-2013

5.1 Harmful Algal Bloom Database

The harmful algal bloom (HABS) database was launched in 2002 and provides access to up-to-date monitoring results of all shellfish toxicity test results collected in Ireland and is a key component of the Irish Shellfish Monitoring Programme.

Data for 2011-2013 were extracted from the database to examine species exposure, percentage of samples above the regulatory limit, time of year and location where high levels were recorded and the range of concentrations found in species. All toxin concentration units are expressed in µg/g, apart from PSP which is µg/100g. All phytoplankton cell counts are expressed in cells/Litre. The data are summarised in the form of graphs and charts and presented for each toxin group in the following chapters.

5.2 *Alexandrium* Species and Paralytic Shellfish Poisoning Toxins

In Irish waters, *Alexandrium* cell counts peak during the summer months, typically from June to July but occurrence ranges from March to September. When an increase in *Alexandrium* cell counts is noted, MI requests analysis of shellfish samples for the presence of paralytic shellfish toxins (PSTs). Thus, all the paralytic shellfish poisoning (PSP) results reported are based on selective testing. However, the presence of *Alexandrium*, even at high cell counts per litre, does not necessarily result in PST production, as both toxic and non-toxic strains occur in coastal waters. The number of shellfish samples analysed therefore, ranges widely from year to year, based on the occurrence of *Alexandrium* in water samples.

In 2011, 22 shellfish samples were analysed but in the following year, in response to higher presence of *Alexandrium*, 106 samples were analysed. This number fell back to 26 samples in 2013. In 2012 and 2013, mussels (*Mytilus edulis*) were the most common shellfish species in which PSTs were detected (56%), followed by Pacific oysters (*Crassostrea gigas)* (37%). Various clam species, whelks (*Buccinium undatum*) and scallops (*Pecten maximus*) made up the remainder.

Figures 7 and 8 provide an overview of presence of *Alexandrium* spp. in Irish waters and corresponding detection of PSTs in shellfish sampled during 2011, 2012 and 2013. The red threshold line in the graphs represents the legal limit above which production areas were closed. No closures were necessary in 2011, whereas in 2012 and 2013, seven and two samples respectively, taken from Cork Harbour were found to be above the threshold, resulting in the closure of the associated production area.

Figure 7. Presence of *Alexandrium* spp. in Irish waters sampled during 2011, 2012 and 2013

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

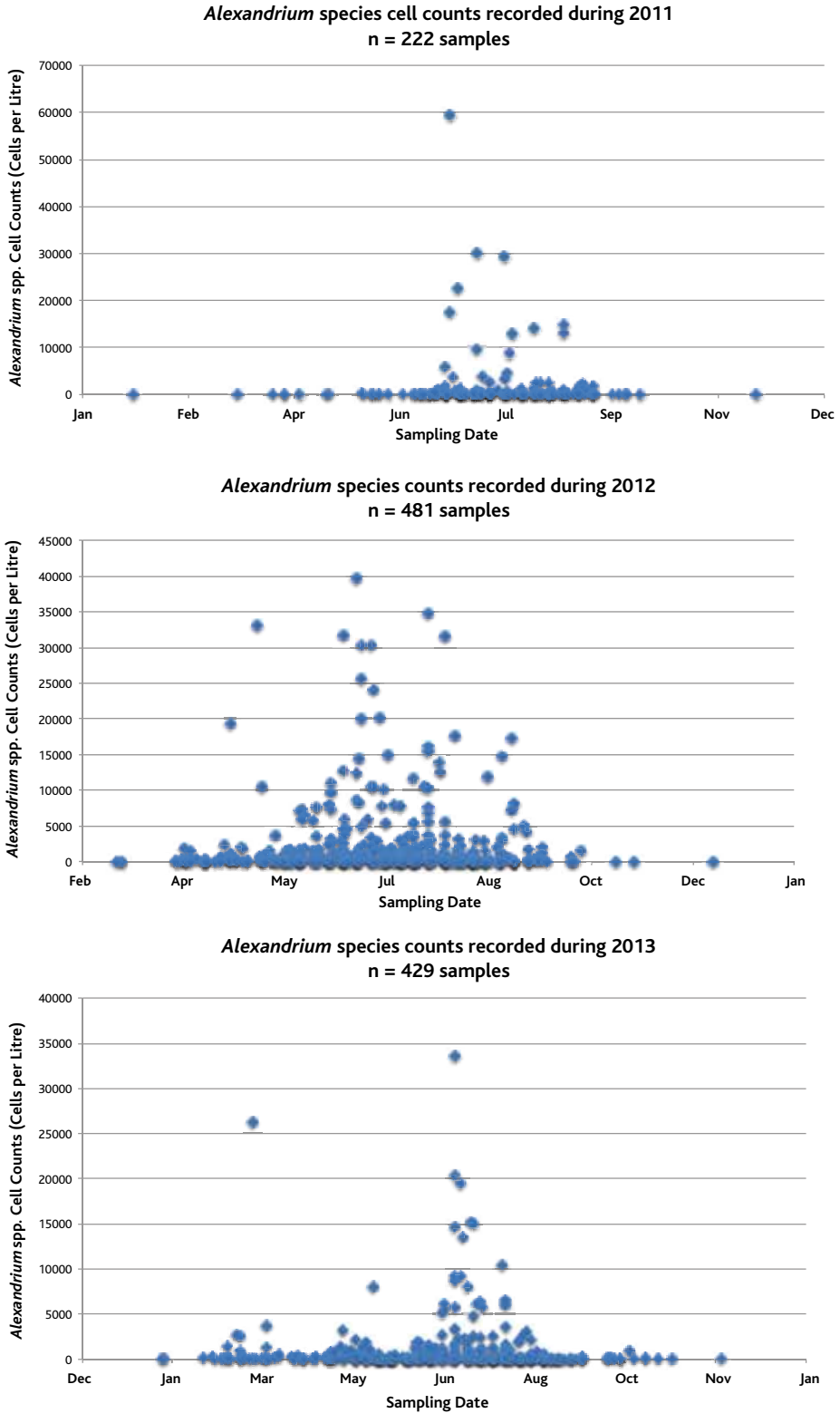
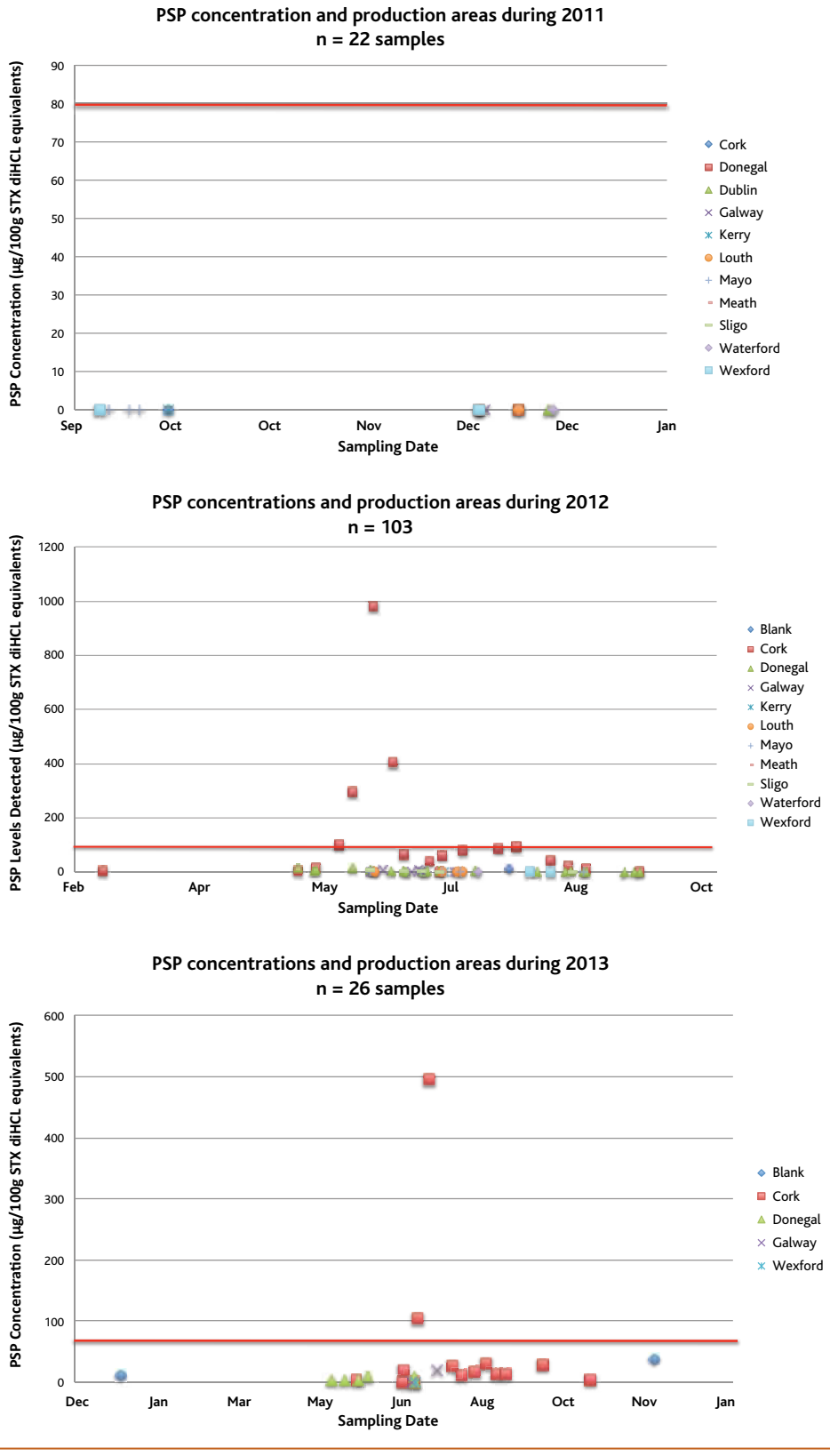


Figure 8. Detection of PSTs in shellfish sampled during 2011, 2012 and 2013

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)



5.3 *Pseudo-nitzschia* species and Amnesic Shellfish Poisoning

The diatom *Pseudo-nitzschia* genus, found commonly in Irish coastal waters, includes species that are known producers of DA, the toxin that can induce ASP. In general, *Pseudo-nitzschia* spp. are grouped into complexes (the '*seriata*' and '*delicatissima*' groups) because most of the species cannot be reliably differentiated using light microscopy. Molecular testing or electron microscopy is usually required to identify each species.

The *Pseudo-nitzschia seriata* complex contains a number of toxic species and while their numbers peak during early to mid-summer, there is a higher risk in spring, when the earliest of these species (including toxic species) occur. At this time of the year, a mono-specific bloom of one particularly toxic species of this group, *P. australis,* can occur, which can cause shellfish to rapidly become toxic and has been implicated in toxic events in Ireland. The generally non-toxic complex *Pseudo-nitzschia delicatissima* typically peaks later in the summer.

Pseudo-nitzschia cell counts begin to increase with the spring bloom during February, March and April, and peak during the summer when they form a significant part of the overall phytoplankton community. As an important food source for shellfish, the monitoring of this genus is essential to prevent accumulation of ASP toxins in shellfish. The mid to late summer *Pseudo-nitzschia* are typically non-toxic and are diluted out among the larger overall summer phytoplankton assemblage. This dilution effect reduces the risk of ASP toxin accumulation in the shellfish.

Figures 9 and 10 provide an overview of *Pseudo-nitzschia* spp. in Irish waters and corresponding detection of ASP toxins in shellfish sampled during 2011, 2012 and 2013. The red threshold line represents the legal limit above which production areas were closed (20 µg/g).

Figure 9. Presence of *Pseudo-nitzschia* sp. in Irish waters sampled during 2011, 2012 and 2013

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

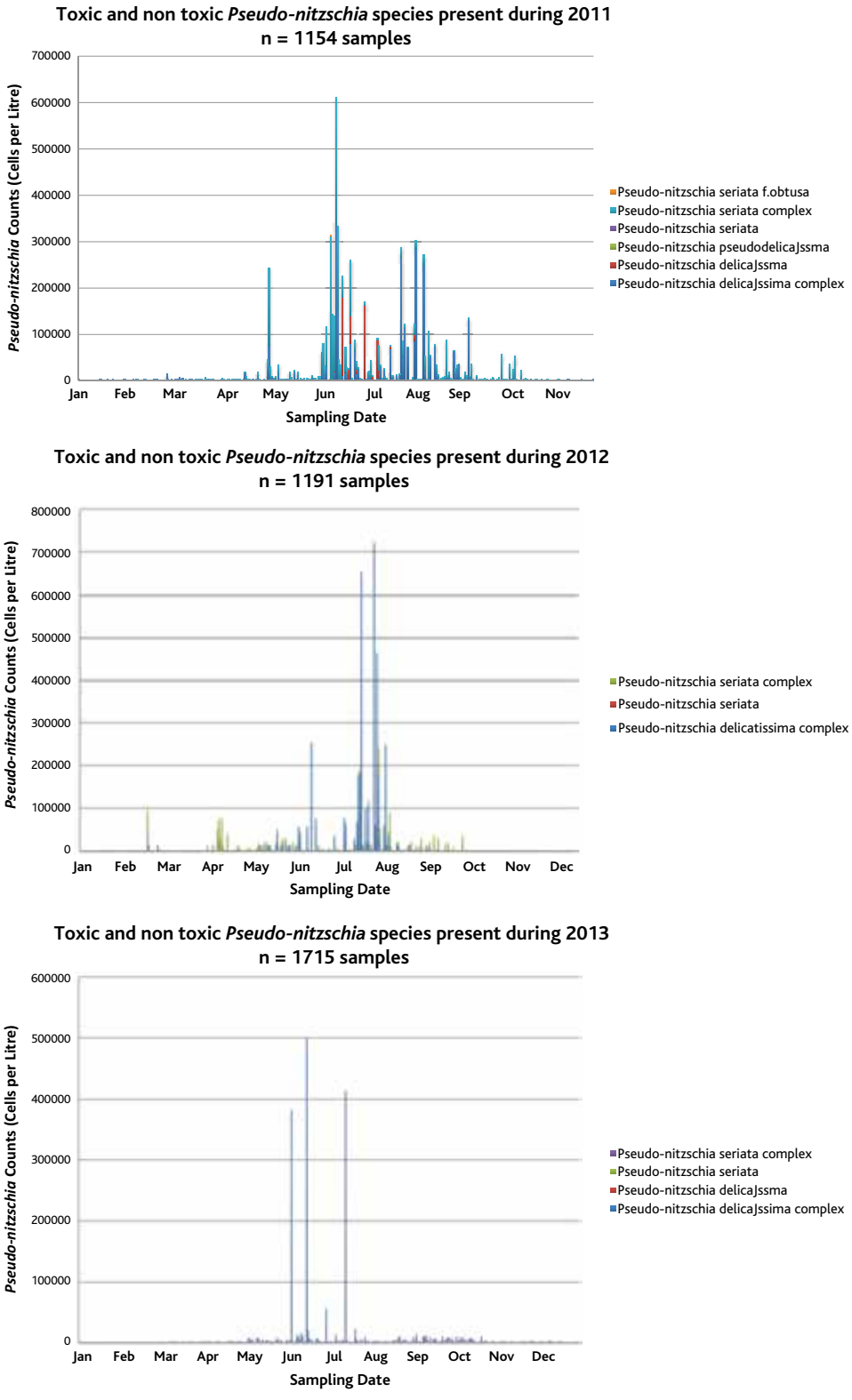
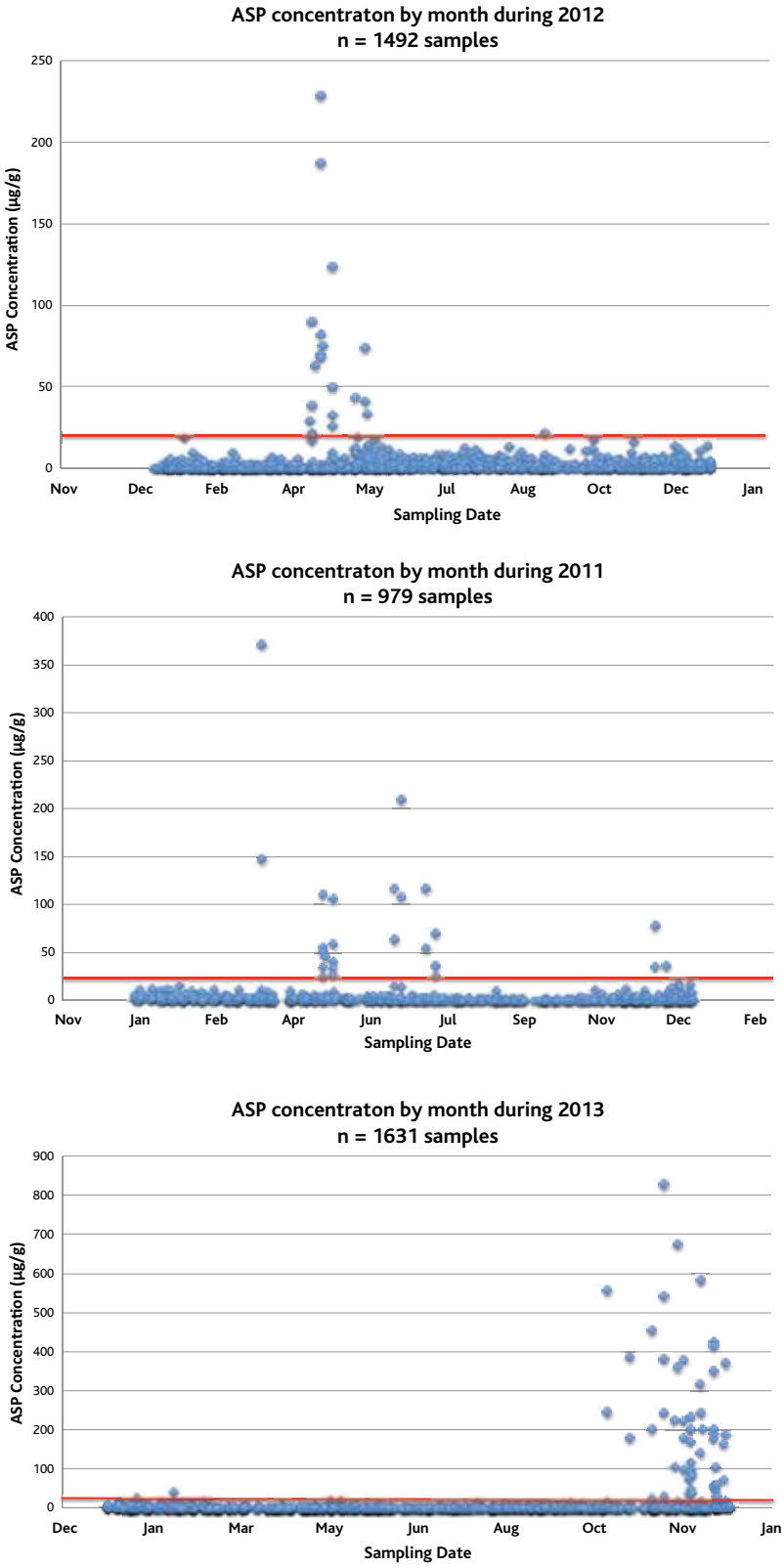


Figure 10. Presence of ASP toxins in shellfish sampled during 2011, 2012 and 2013

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)



There were six distinct *Pseudo-nitzschia* species present in Irish waters in 2011: *Pseudo-nitzschia seriata f.obtusa*, the *seriata* complex, *P. seriata*, *P. pseudodelicatissma*, *P. delicatissma*, the *P. delicatissima* complex. *P. seriata* complex peaked in June; *P. delicatissima* complex had a smaller peak in August.

This contrasted with only three distinct species detected in 2012: *Pseudo-nitzschia seriata* complex, *P. seriata*, and the *P. delicatissima* complex. There was small peak of *P. seriata* complex in April and May, followed by a larger *P. delicatissima* complex peak in July and August.

There were four distinct species present in 2013: *P. seriata* complex, *P. seriata*, *P. delicatissma*, and the *P. delicatissima* complex. There were only peaks of *Pseudo-nitzschia delicatissima* complex, these occurred in June and July.

ASP toxin outbreaks do not always correlate with large *Pseudo-nitzschia* peaks, rather the time of year when the peaks occur is of importance. ASP toxin peaks often correlate with *Pseudo-nitzschia seriata* and *Pseudo-nitzschia seriata* complex spring bloom. This is due to the toxic species *Pseudo-nitzschia australis* being a component of the *seriata* complex. This was seen in 2011 when ASP toxin concentrations above the regulatory limit peaked between April and July. Twenty-five samples were above the regulatory limit (2.55% of total samples).

In 2012, ASP toxin concentrations above the regulatory limit peaked between April and May. Twenty-one samples were above the regulatory limit (1.41% of total samples).

In 2013, there was very little *P. seriata* or *P. seriata* complex recorded. *P. delicatissma* complex had a late bloom in June. Fifty-seven samples were above the regulatory limit (3.49% of total samples). The bulk of these were scallops which were toxic above regulatory levels in the latter half of the year. The typical spring/early summer toxicity in mussels was limited to a single sample that went above regulatory levels during the spring.

From 2011 to 2013, scallops (*Pecten maximus*) and mussels (*Mytilus edulis*) were the most common species in which ASP toxins were isolated (see Table 5).

Table 5. Percent of species in which ASP toxins were isolated

Species	Percent of samples in which ASP toxins were isolated		
	2011 (N=979)	**2012 (N=1,492)**	**2013 (N=1,631)**
Scallops (*Pecten maximus*)	84	68	77
Mussels (*Mytilus edulis*)	8	17	12

Looking at the longer term distribution of ASP toxin concentrations in the non-scallop species, peaks were recorded in spring of 2005 and 2009, with smaller peaks recorded in 2010, 2011 and 2012 (see Figure 11). All of the significant outbreaks in mussels were recorded between latitudes 51°N - 52°N.

There was a variation in the concentration range and species in which ASP toxins were recorded between 2011 and 2013 (see Figure 12). The greatest range occurred in King Scallops (*Pecten maximus*) in 2011 and 2013, the same years for which the greater number of *Pecten maximus* samples were above the regulatory limit. In 2012, *Mytilus edulis* showed the greatest range in values and exceeded the regulatory limit (20 µg/g). A possible reason for the huge variation seen in scallops is that the tissue submitted for testing is very variable, i.e. for inshore testing the whole animal tissue is analysed whereas for offshore testing, only shucked produce, i.e. muscle and gonads, are submitted.

Figure 11. Distribution and concentration (µg DA equivalents per gram) of ASP in Irish bivalves (non-scallop) species from 2002 to 2012

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

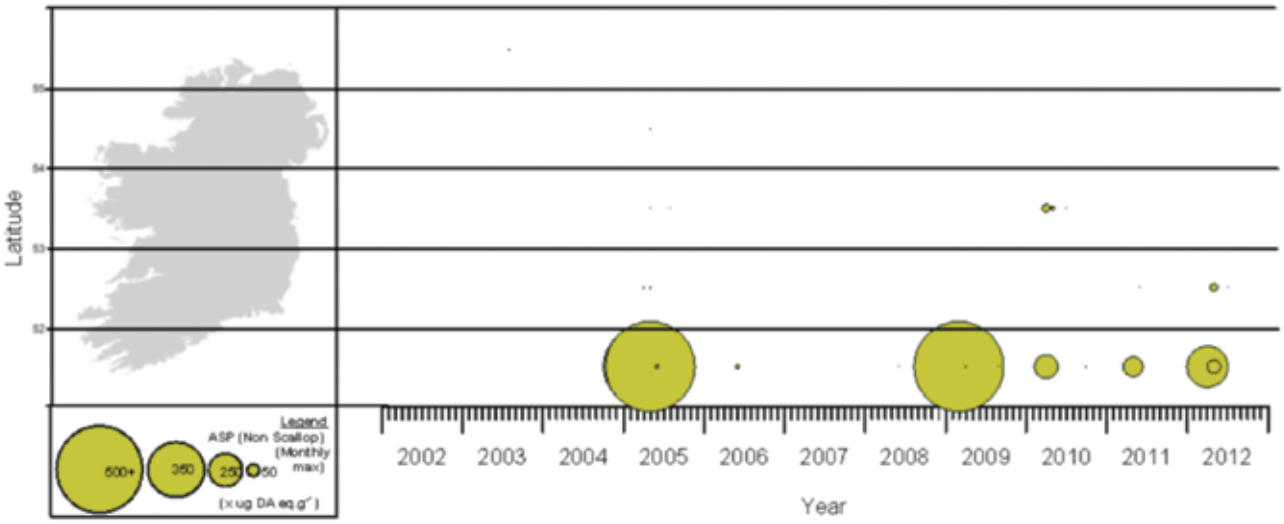
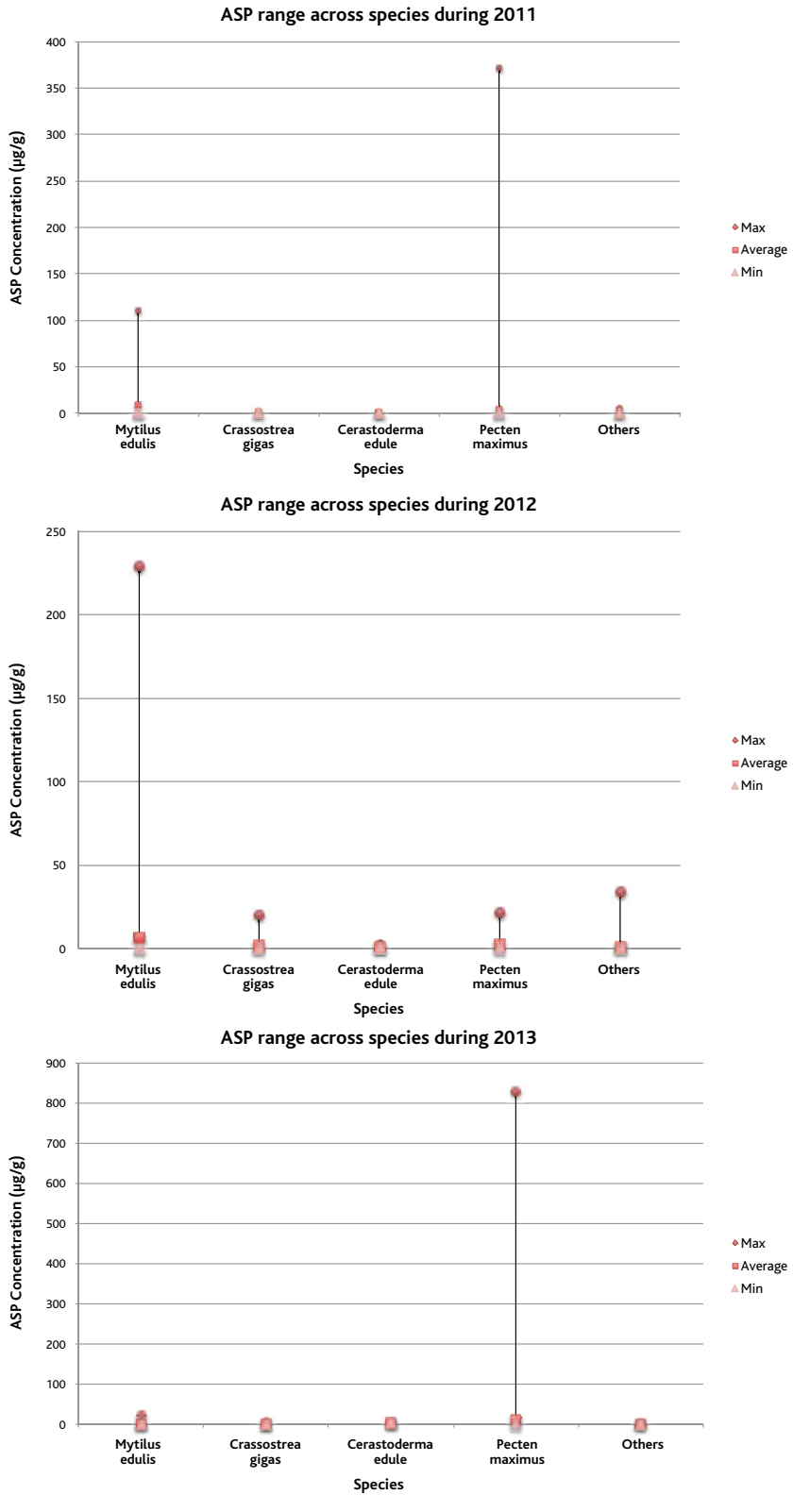


Figure 12. Range of ASP concentrations (maximum, average and minimum) recorded during 2011, 2012 and 2013 across the species

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)



5.4 *Dinophysis* species and Diarrhetic Shellfish Poisoning, Yessotoxins, Pectenotoxins

Dinophysis are a marine dinoflagellate phytoplankton group, which can cause the accumulation of DSP toxins in filter feeding shellfish. The causative toxins are OA, *Dinophysis* Toxins (DTX) and Pectenotoxins (PTXs). Another group of toxins, the YTXs are produced by the dinoflagellate species *Lingulodinium polyedrum* (among others, see section 2.1.3). The toxins from *Dinophysis* and *Lingulodinium* are found in Irish shellfish however, *Lingulodinium polyedrum* is rare in Irish waters, occasionally blooming in short acute bursts in mid-summer, but not to particularly high levels. The resulting occurrence of YTX is similarly rarely observed, and only at very low levels in shellfish. PTX has been detected occasionally as a minor co-occurring toxin along with the other DSP/DTX toxins

In general, *Dinophysis acuminata* increases in numbers from the end of April start of May, resulting in increasing levels of OA. *Dinophysis acuta* increases in concentration later in summer resulting in DTX2 production. This was the case in 2011 and 2013 however, in 2012 there was no *Dinophysis acuta* peak later in the year. Figures 13 and 14 provide an overview of *Dinophysis* sp. occurrence in Irish waters and corresponding detection of DSP toxins in shellfish sampled during 2011, 2012 and 2013. The red threshold line represents the legal limit above which production areas were closed (0.16 µg/g expressed as OA equivalents).

In 2011, there were nine distinct *Dinophysis* species detected: *Dinophysis acuminata*, *Dinophysis acuta*, *Dinophysis caudata*, *Dinophysis dens*, *Dinophysis fortii*, *Dinophysis nasutum*, *Dinophysis norvegica*, *Dinophysis tripos* and an unidentified *Dinophysis* species.

Dinophysis acuminata peaked between May and July, *Dinophysis acuta* peaked between August and October. Out of 402 samples, *Dinophysis acuminata* was most common (60%), followed by *Dinophysis acuta* (28%). Eighty samples of shellfish were above the regulatory DSP toxins limit (4.60% of all samples). Figure 13 illustrates the bimodal distribution that occurs during the year, the first peak between the end of April and start of May was due to *Dinophysis acuminata* counts rising and resulted in OA production. The second peak from July to November reflected the increase in *Dinophysis acuta* and resulted in DTX2 production.

In 2012, there were seven distinct *Dinophysis* species detected: *Dinophysis acuminata, Dinophysis acuta, Dinophysis dens, Dinophysis fortii, Dinophysis odiosa, Dinophysis* spp. indet and *Dinophysis tripos*.

Out of 584 phytoplankton samples in which *Dinophysis* were identified, *Dinophysis acuminata* was most common (75%), followed by *Dinophysis acuta* (14%). *Dinophysis acuminata* peaked between May and July; there was no distinct *Dinophysis acuta* peak later in the year, it appeared earlier and was mixed in with the *Dinophysis acuminata* bloom. Consequently, there is no bimodal DSP toxins distribution observed in the shellfish in 2012. Of the 2,390 shellfish samples analysed, 52 samples were above the regulatory limit (2.18%) with a single peak between May and August.

In 2013, there were four distinct *Dinophysis* species detected: *Dinophysis spp. indet*, *Dinophysis dens*, *Dinophysis acuta*, *Dinophysis acuminata*.

Dinophysis acuminata peaked between May and July; and a more seasonal *Dinophysis acuta* peak was detected between August and October. Out of 462 phytoplankton samples, *Dinophysis acuminata* was most common (69%), followed by *Dinophysis acuta* (19%). Forty-two shellfish samples were above the regulatory limit (1.70%). Figure 13 illustrates the bimodal distribution that occurred during that year. The first peak began in May due to *Dinophysis acuminata* counts rising and resulted in OA production. The second peak from June to October reflected the increase in *Dinophysis acuta* and resulted in DTX2 production.

Figure 13. Presence of *Dinophysis* spp. in Irish waters during 2011, 2012 and 2013

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

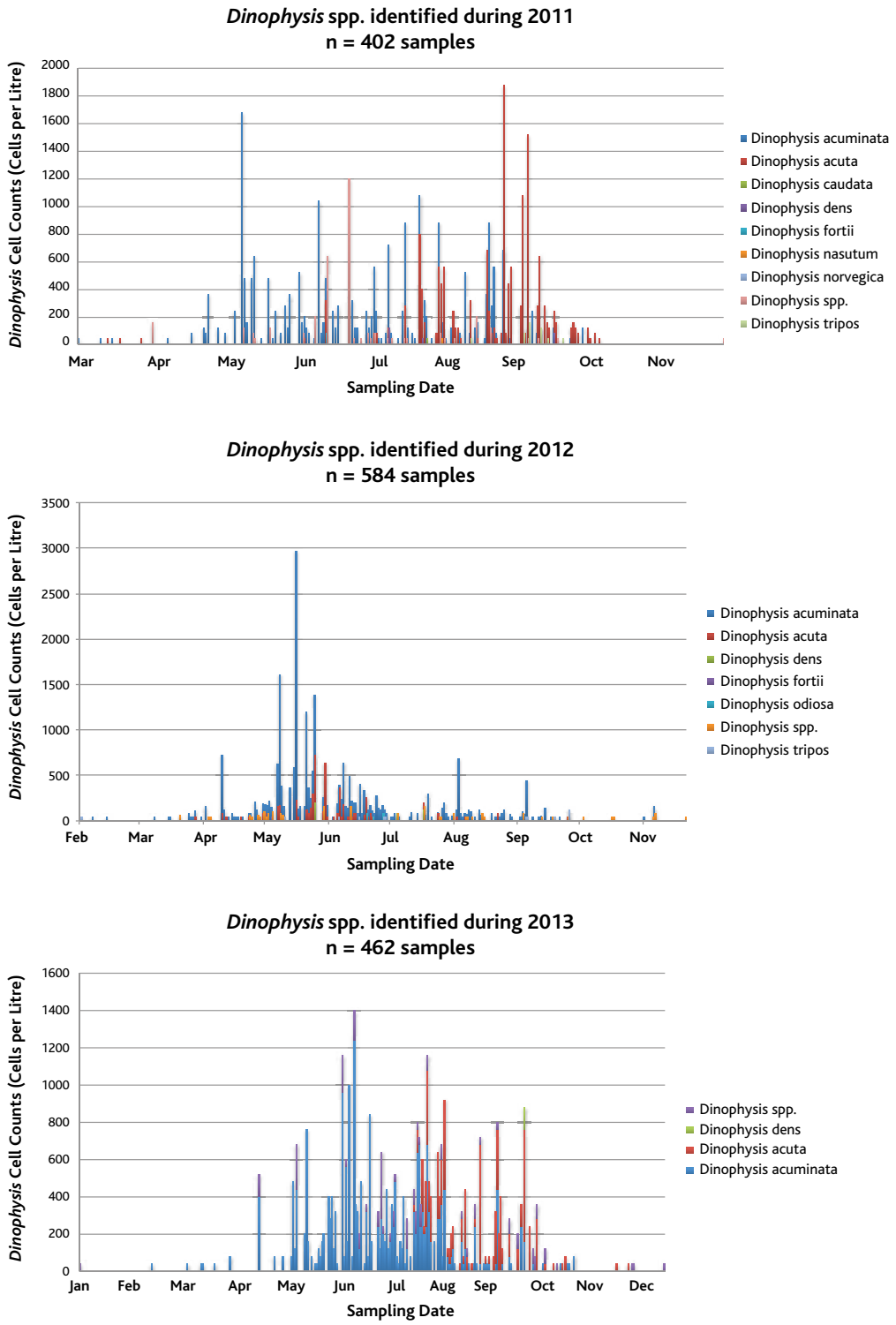


Figure 14. Presence of DSP toxins in shellfish sampled during 2011, 2012 and 2013

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

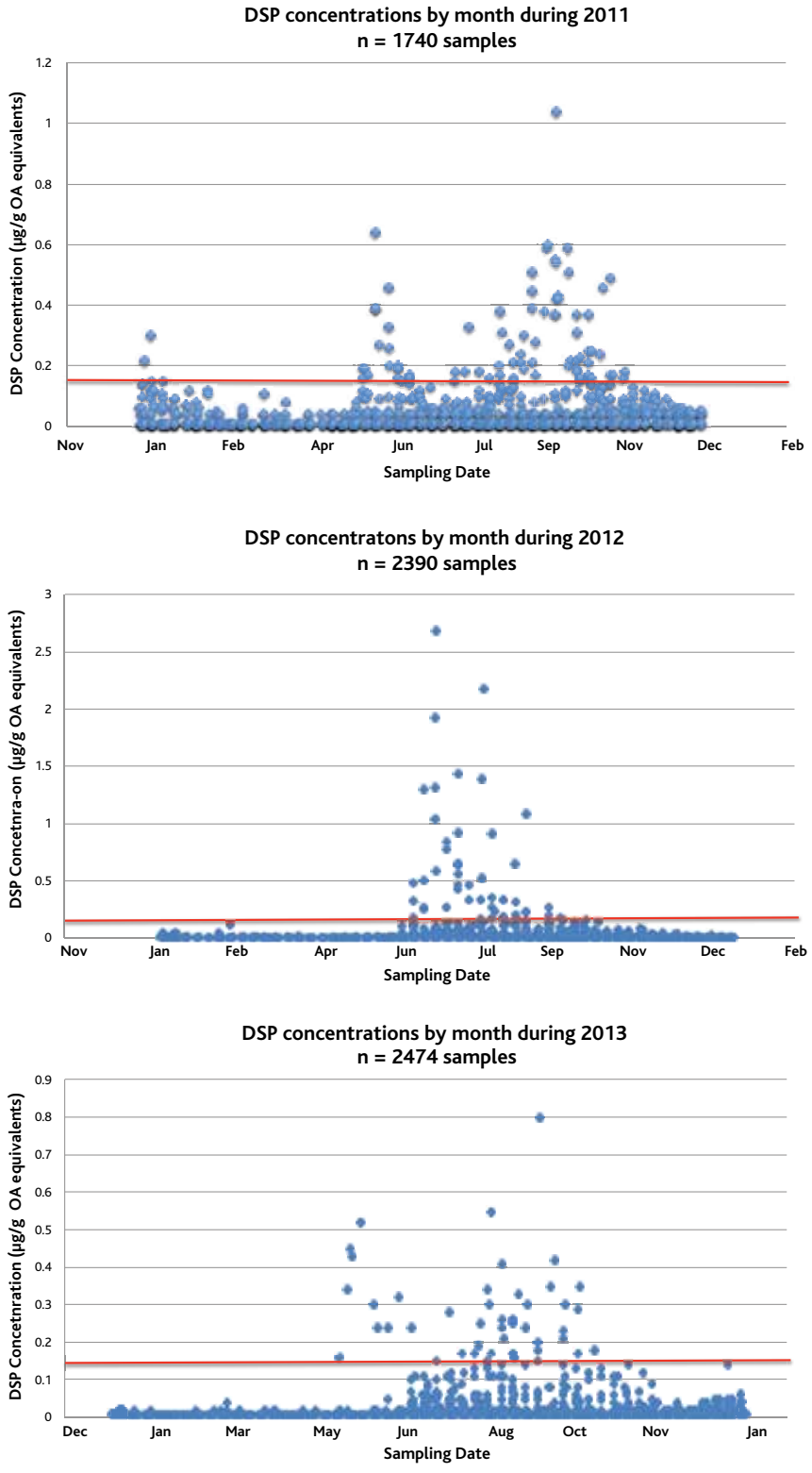
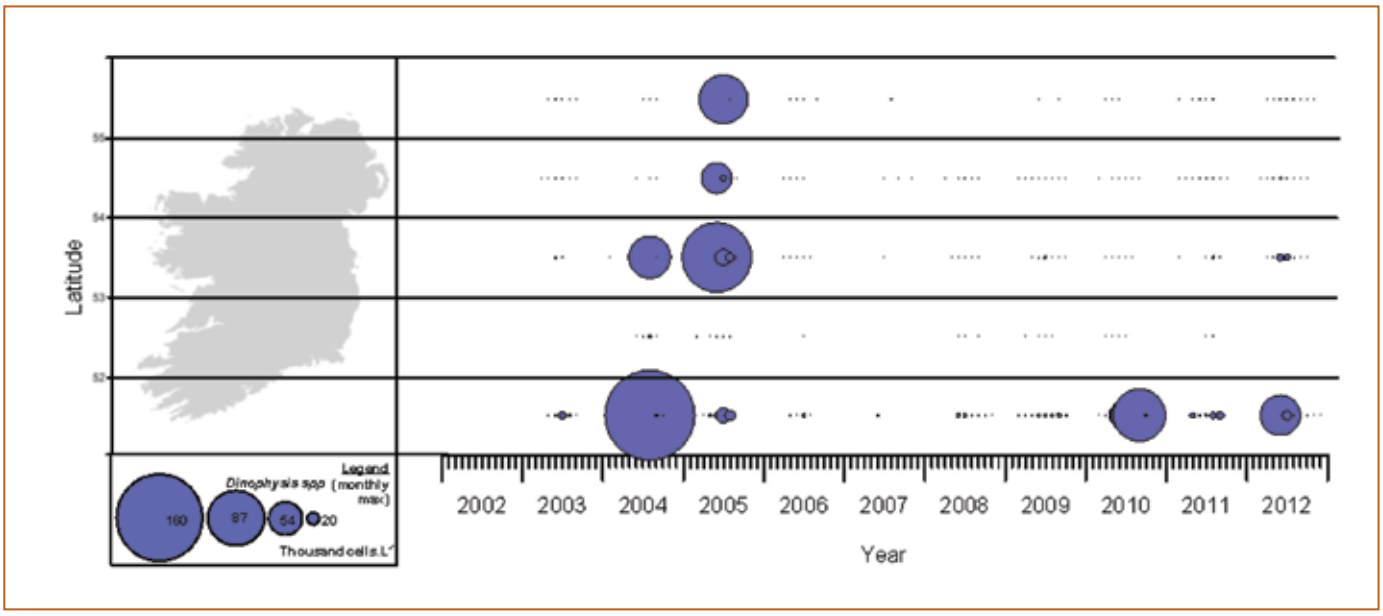


Figure 15. *Dinophysis* species distribution and concentration (thousand cells per litre) from 2002 to 2012

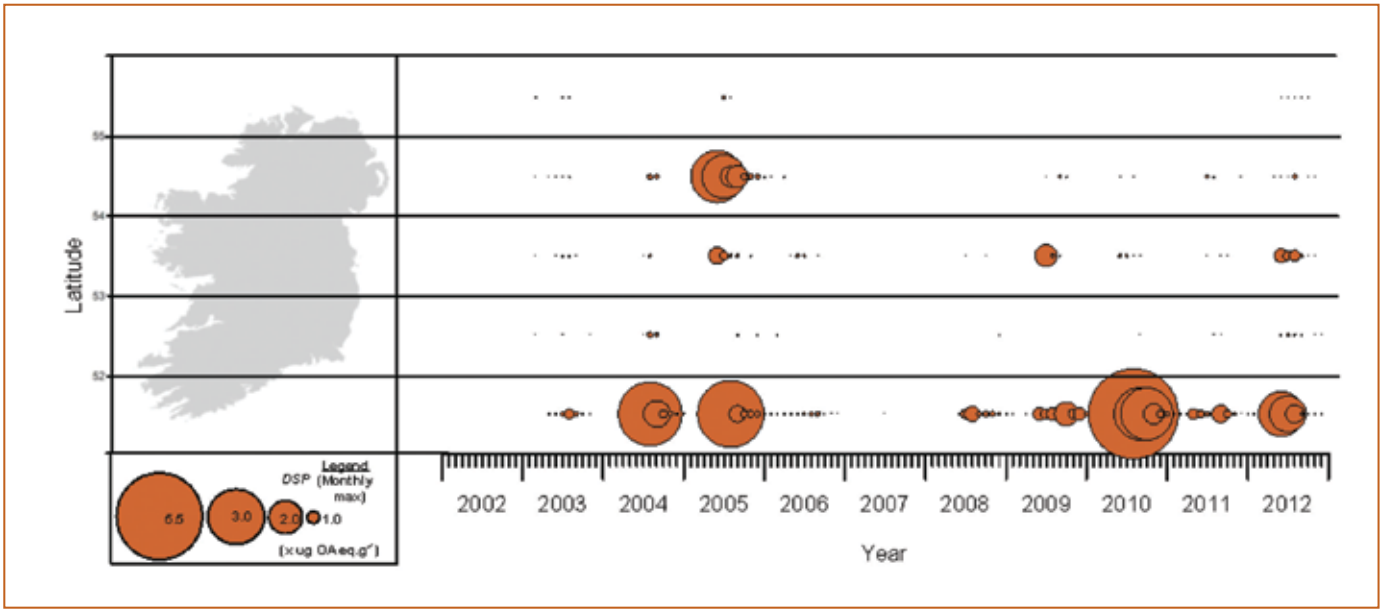
(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)



Dinophysis is commonly found in all coastal waters and is present every year in various concentrations, usually up to a maximum of tens of thousands of cells. It does occasionally exceed this and Figure 15 shows the concentration and distribution of the species between 2002 and 2012. It peaked up to 160 thousand cells/L during 2004 between latitudes 51°N - 52°N and 53°N - 54°N. In 2005, there were other smaller peaks between latitudes 53°N -54°N, 54°N - 55°N and 55°N - 56°N. In 2010 and 2012, peaks occurred between latitudes 51°N - 52°N.

Figure 16. DSP toxins distribution and concentration (µg OA equivalents per g)

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

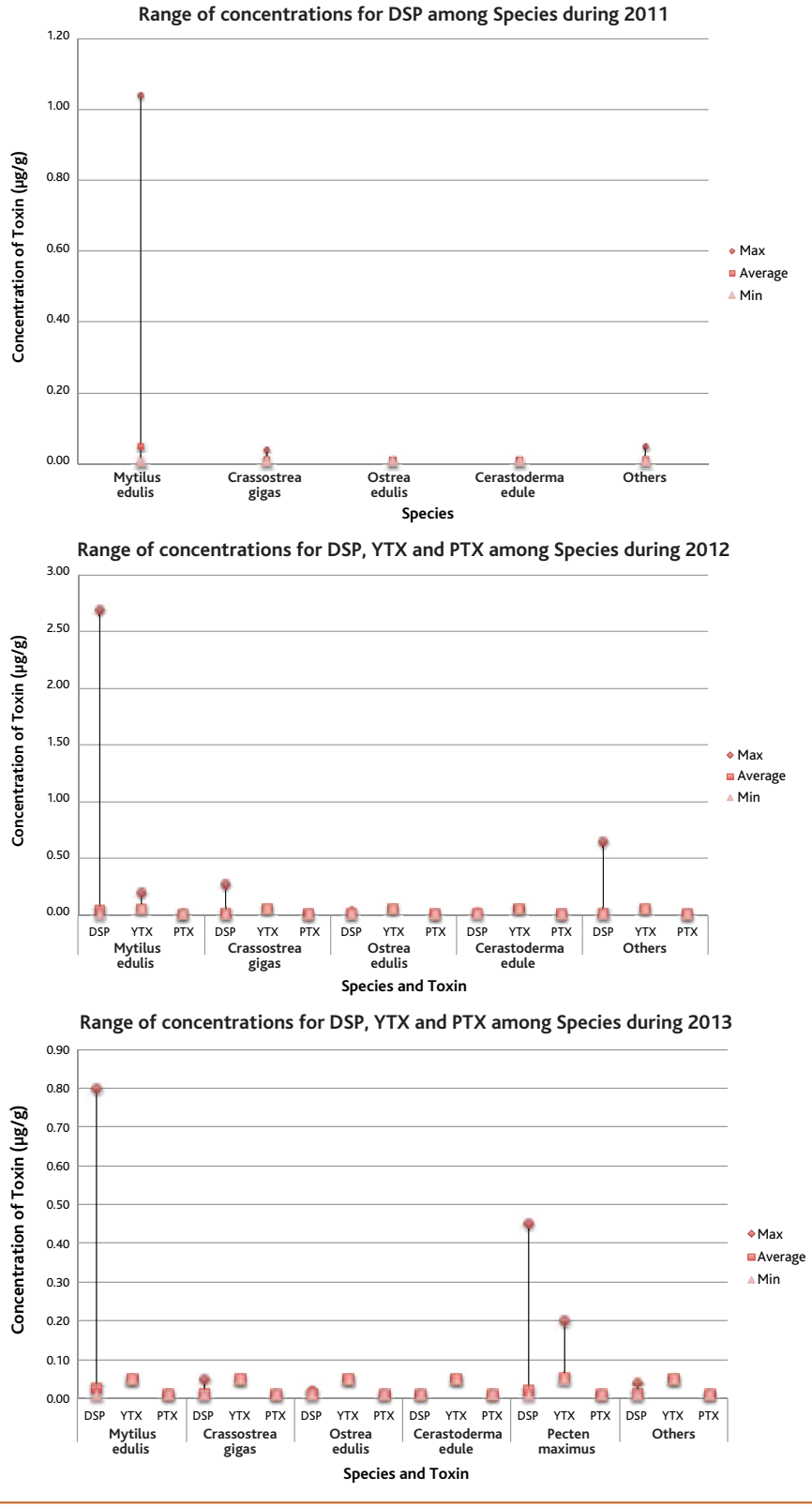


Corresponding large DSP toxins concentrations were recorded (see Figure 16) in 2004, 2010 and 2012 between latitudes 51°N -52°N and in 2005 between latitudes 51°N - 52°N and 54°N - 55°N. From a risk assessment aspect, DSP toxins mainly cause problems in mussels (*Mytilus edulis*). DSP toxins distribution tends to occur in the southern half of Ireland as this is where the greatest number of mussels (*Mytilus edulis*) farms are located. Mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas)* were the most common species in which DSP toxins were detected in the study period however, the toxins have been found in all bivalve species on occasion.

The range of concentrations for DSP toxins was greatest in mussels (*Mytilus edulis*), the most common species in which the toxin occurs (Figure 17). The levels of DSP toxins (OA/DTX and their esters), YTX and PTX may be compared for the years 2012 and 2013 only; no YTX or PTX was detected in 2011. This comparison illustrates that YTX and PTX concentrations were far below the regulatory limits (3.75 µg/g and 0.16 µg/g respectively). In 2013, DSP toxins showed the greatest variation in mussels (*Mytilus edulis*) and scallops (*Pecten maximus*) species and exceeded the regulatory limit. While YTX also showed some variation in scallops (*Pecten maximus*) species, both YTX and PTX did not exceed the regulatory limits.

Figure 17. Range of concentrations (maximum, average and minimum) recorded across the species for DSP toxins (OA/DTX and their esters), YTX and PTX during 2011, 2012 and 2013

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)



5.5 Azaspiracids

Azaspiracid (AZA) concentrations increase during the summer and autumn. There were much higher peaks in 2012 (7.5 µg/g) and 2013 (3.35 µg/g) compared to 2011 (0.27 µg/g) due to a higher concentrations of the causative phytoplankton (*Azadinium*) during the summer. This toxicity lasted through the autumn months and into the winter in a number of areas and persisted into January and February of the following years, but dissipated with the onset of the spring-bloom in March and April. Figure 18 provides an overview of AZA toxin occurrence in shellfish sampled during 2011, 2012 and 2013. The red threshold line represents the legal limit above which production areas were closed (0.16 µg/g AZA-1 equivalents).

In 2011, AZA concentrations peaked between July and November, reaching a maximum of 0.27 µg/g. Six samples were above the regulatory limit (0.35% of all AZP samples). The following year, AZA concentrations peaked between June and October, reaching a maximum of 7.5 µg/g, far greater than 2011 and 2013. 427 samples were above the regulatory limit (17.9% of all AZA samples) in 2012. In 2013, AZA concentrations peaked later after July and up to the end of the year, reaching a maximum of 3.35 µg/g. 220 samples were above the regulatory limit (8.84% of all AZA samples) in 2013.

Figure 18. Presence of azaspiracid toxins in shellfish sampled during 2011, 2012 and 2013

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

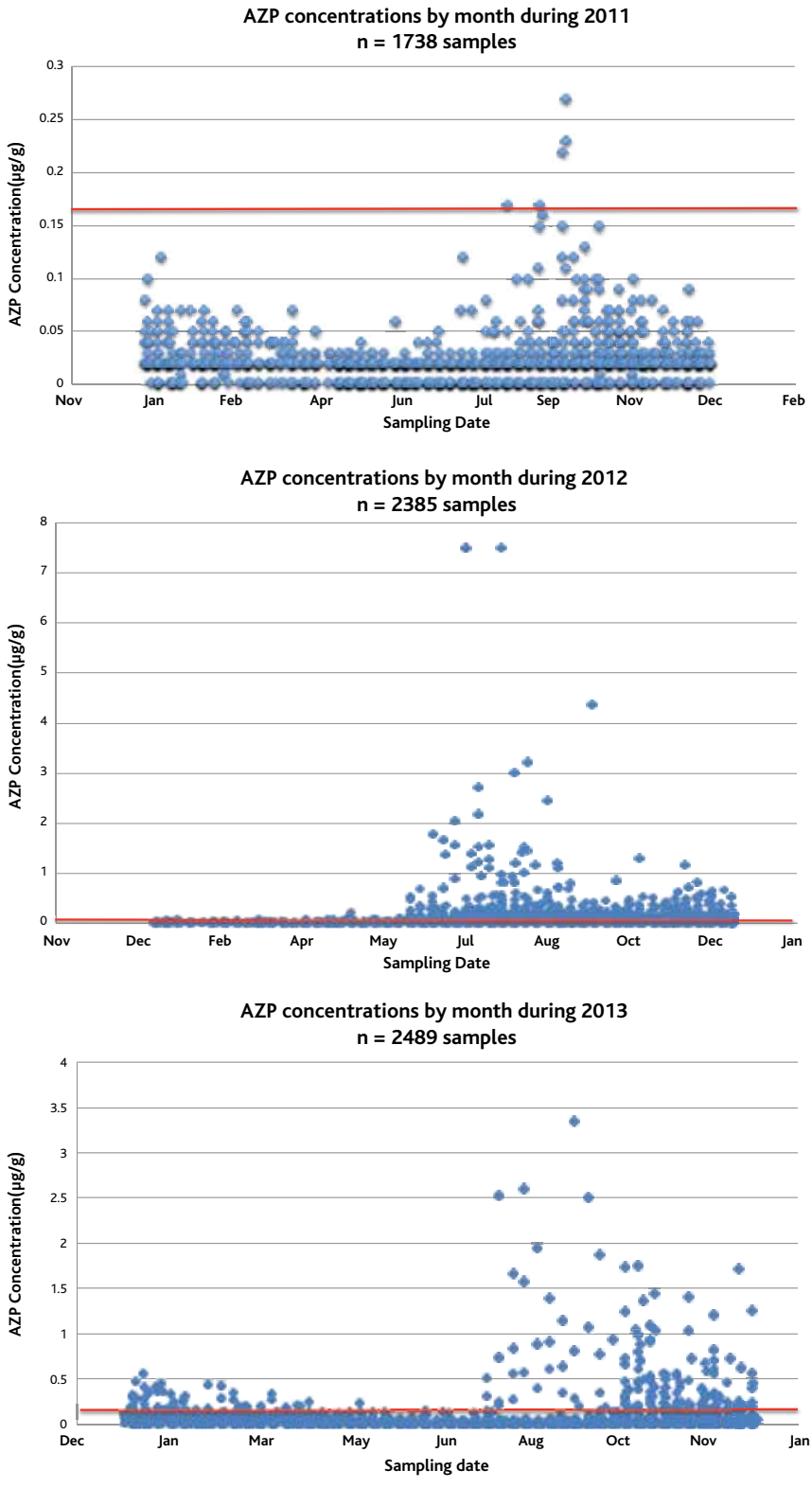


Figure 19. Azaspiracid distribution and concentration (AZA µg equivalents per gram) in Irish farmed mussels (*M. edulis*) between 2002 and 2012

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)

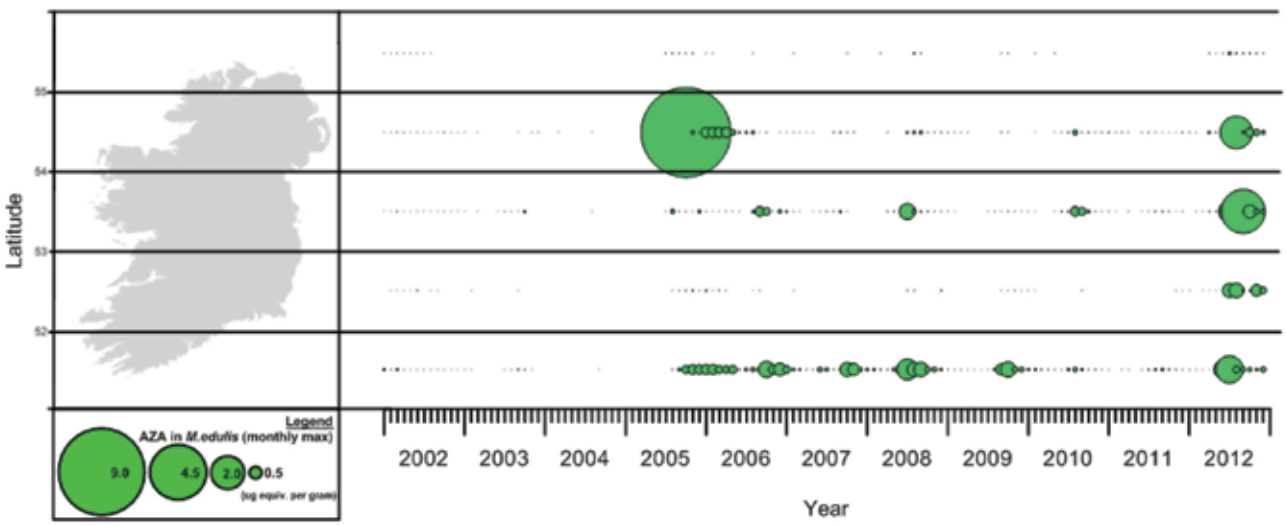


Figure 19 shows the annual occurrence between 2002 and 2012 of AZA, and the geographic distribution. While AZA is found nationwide, the south is most impacted by this toxin due to its high occurrence in mussels that are predominant aquaculture crops in this region. In 2005 there was a significant peak further north between latitudes 54°N - 55°N. In 2012 there were many smaller peaks between latitudes 51°N - 52°N, 52°N - 53°N, 53°N - 54°N and 54°N - 55°N. From 2011 to 2013, the proportion of AZA samples over the regulatory limit varied from 0.35% in 2011, 17.87% in 2012 and 8.84% in 2013 (Table 6). The very high percentage in 2012 correlates with the extremely high peak AZA concentration in the same year (7.5 µg/g, Figure 18). Similarly, the low percentage in 2011 corresponds to a very low maximum AZA concentration (0.27 µg/g).

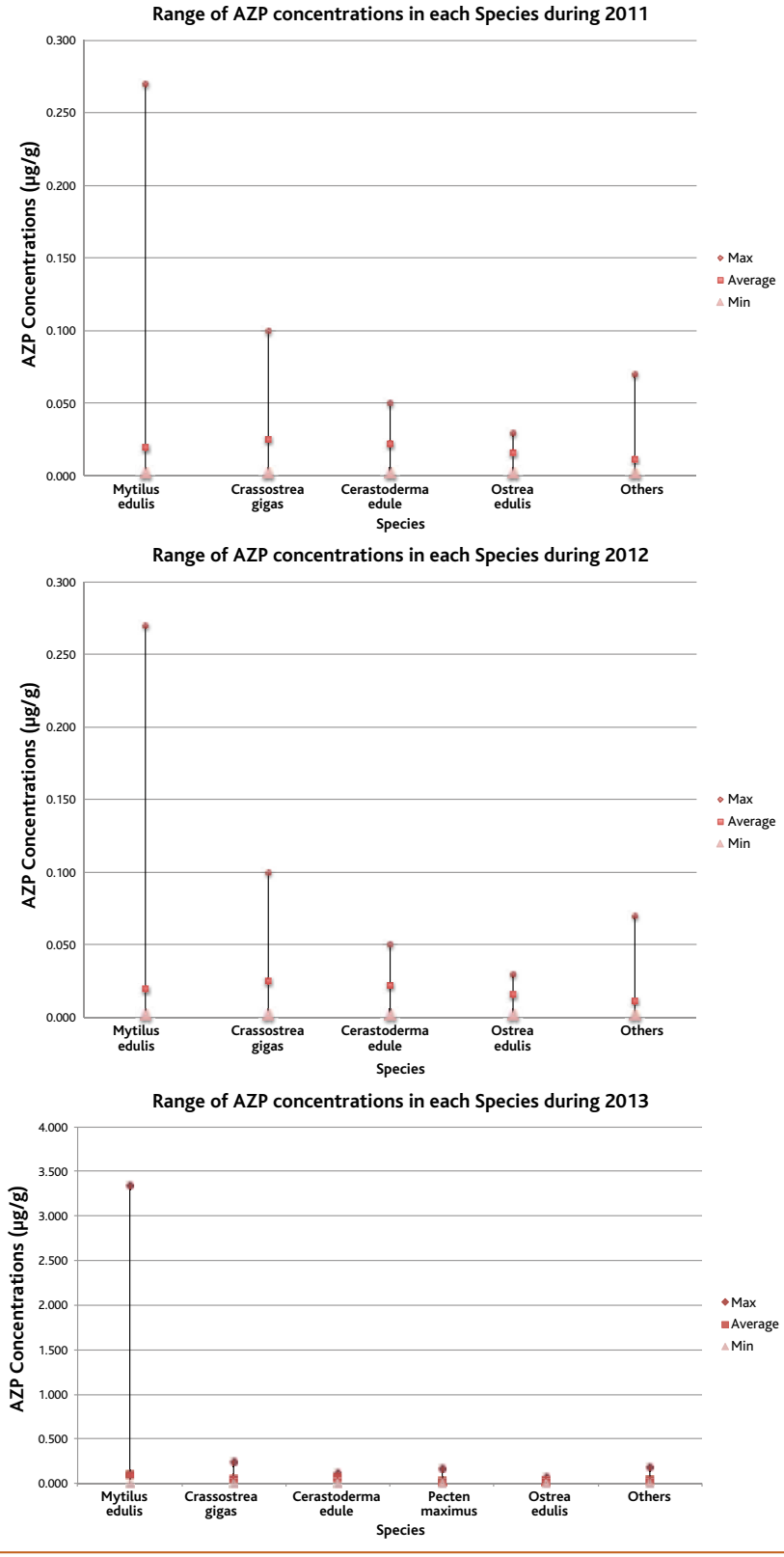
Table 6. Summary of azaspiracid poison (AZP) samples, concentration, the number and percentage of samples over the regulatory limit

Year	Total Number of AZP Samples	Peak AZP Concentration (µg/g)	Total Number of AZP Samples over Regulatory Limit	% of AZP Samples over Regulatory Limit
2011	1,738	0.27	6	0.35%
2012	2,389	7.5	427	17.87%
2013	2,489	3.35	220	8.84%

There are large mussel industries in counties Cork and Galway. This is reflected in locations where AZA was recorded over the regulatory limit. In 2011, AZA was most commonly found in Co. Cork production areas in the period between July and October. In 2012, AZA was most commonly found in Co. Galway between August and October but was also present at two other locations in Co. Cork (July) and Co. Donegal (August). In 2013, AZA was most commonly found in Co. Galway between July and October, followed by Co. Cork between October and December.

Figure 20. Range of AZA concentrations (maximum, average and minimum) recorded during 2011, 2012 and 2013 across the species

(Source: Review of Novel Marine Biotoxins in Irish Shellfish, Eithne Mac Carthy M.V.B., 2014)



The greatest range of AZA values is found in the species in which AZA was most commonly detected, mussels (*Mytilus edulis*), for each of the years between 2011 and 2013 (Figure 20). Mussels (*Mytilus edulis*) was also the species in which AZA was most commonly detected, followed by oysters (*Crassostrea gigas)*. In terms of the regulatory limit (0.16 µg/g), only for mussels (*Mytilus edulis*) did the maximum value exceed this limit in 2011 for all five species categories the maximum values exceeded this limit in 2012, and for the species categories mussels (*Mytilus edulis*), oysters (*Crassostrea gigas)* and scallops (*Pecten maximus*), the maximum values exceeded this limit in 2013.

CHAPTER 6. EXPOSURE ASSESSMENT

Consumption of seafood, as recorded in the National Adult Nutrition Survey (NANS) (IUNA, 2011), was combined with the legislative limits for marine biotoxins to produce a theoretical regulatory maximum level exposure assessment scenario for the adult population resident in Ireland. Results were assessed against the Acute Reference Dose (ARfD) set by EFSA for each toxin.

6.1 Food Consumption Database

The NANS was conducted between 2008 and 2010 by the Irish Universities Nutrition Alliance (IUNA, 2011). This survey provided data on 1,500 adults (18-90 years of age) for food, beverage and nutritional supplement intake along with habitual physical activity levels, attitudes to food and health and factors influencing food choice. Physical measurements (including weight, height, body fat and blood pressure) were also taken. Four day, semi-weighed food diaries were used to record the food and drink intake of the participants. Each time food/drink was consumed, it was recorded as well as the location, amount, cooking method and quantity of each food item/drink consumed. All data collected were stored in SPSS databases. The primary food consumption file (food file) provides food intake on an individual level, with each individual line in the file representing one single eating occasion recorded by the participant (see Bivalve Mollusc Eating Occasions - Annex, Table 11).

6.2 EU Limits and ARfDs set by EFSA

Maximum levels (MLs) for marine biotoxins in live bivalve molluscs are laid down in Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. ARfDs for okadaic acid and analogues (EFSA, 2008b), azaspiracid (AZA)-group toxins (EFSA, 2008a), saxitoxin (STX)-group toxins (EFSA, 2009b), pectenotoxin (PTX)-group toxins (EFSA, 2009a) and domoic acid (DA) (EFSA, 2009c) were established by EFSA in the years 2008 and 2009.

Table 7 provides a summary of regulatory limits and corresponding ARfDs for each toxin group used in the exposure assessment.

Table 7. Legislative MLs (expressed as toxin equivalents per kg shellfish meat) and ARfDs (expressed as toxin equivalents per kilogram bodyweight)

Toxin Group	Toxin	Current EU ML	ARfD
PSP/Saxitoxin group (Hydro)	STX	800 µg PSP eq./kg SM[1]	0.5 µg STX eq./kg bw
DSP/Okadaic acid group (Lipo)	OA and analogues	160 µg OA eq./kg SM[1]	0.3 µg OA eq./kg bw
PTX /Pectenotoxins group (Lipo)	PTX	160 µg OA eq./kg SM[1]	0.8 µg PTX2 eq./kg bw
ASP/Domoic Acid group (Hydro)	DA	20 mg DA eq./kg SM[1]	30 µg DA eq./kg bw
AZP/ Azaspiracids group (Lipo)	AZA	160 µg AZA eq./kg SM[1]	0.2 µg AZA eq./kg bw

[1] SM – shellfish meat

With the exception of scallops, bivalve molluscs are generally tested on a single whole-tissue basis. For off-shore scallops, MI receives the shucked product, i.e. muscle meat and gonads, whereas for in-shore scallops, muscle meat, gonads and remainder tissue (including the hepatopancreas) are tested individually with a total tissue calculated result determined based on the three tissue results. Depending on the type of toxin, the hepatopancreas is sometimes screened first to determine if all three tissues require testing.

6.3 Influence of Processing on Shellfish Toxin Concentration

The MLs listed in Table 7 have been established for the control of live bivalve molluscs. In 2009, EFSA reviewed information on the influence of processing on the toxin concentration in shellfish (EFSA, 2009e). Several-fold increases in toxin concentration were observed due to water loss during cooking, e.g. up to 80% in the case of OA and therefore, for the purposes of the exposure assessment, shellfish portion sizes have been corrected for weight loss and are expressed as raw weight.

However, EFSA also found some evidence that conversion between toxin analogues can take place, which can result in an increase of certain toxins, such as the conversion of AZA17 into AZA3. For other shellfish toxins, such as YTX and PTXs, no such information exists but there is no reason to assume that they would be different to other lipophilic marine biotoxins.

A further influencing factor on shellfish toxin concentration in the processed product is redistribution of OA-group toxins from the digestive gland to the remaining tissues during processing. This indicates that the analysis of whole shellfish flesh is appropriate for regulatory purposes, particularly when processed shellfish are analysed. Consequently, since cooking can lead to an increase in the levels of lipophilic marine biotoxins in shellfish meat, there is a need for harmonisation of sample pre-treatment practices, i.e. cooking versus non-cooking, before the actual analysis of lipophilic marine biotoxins is carried out (EFSA, 2009e).

6.4 Exposure Assessment: Adults in Ireland

Information on consumption of bivalve molluscs (including use as an ingredient) was extracted from the NANS database and exposure estimates are provided for the adult population resident in Ireland. Since marine biotoxins are acutely toxic (data on chronic toxicity is lacking), acute exposure, i.e. single eating occasions rather than long term average consumption, are of interest. Therefore, the NANS database was interrogated to provide estimates for the typical average portion size and also for the maximum portion size across all eating occasions of bivalve molluscs recorded during the survey period.

Intake estimates are provided for the edible portion (after correction for the shell part) of mussels, oysters and other bivalves, expressed as total portion weight (grams) and portion weight (grams)/kg bodyweight. All portion sizes have been corrected for weight loss due to cooking and are expressed as raw weight (Table 8).

Table 8. Average and maximum Irish portion sizes of bivalve molluscs expressed in grams raw weight/eating occasion and grams raw weight/kg bw/eating occasion as recorded in NANS (see Annex I for full details)

Bivalve Mollusc	Eating Occasions	Average Portion g	Maximum Portion g	Average Portion g/kg bw	Max Portion g/kg bw
COCKLES	1	119.4	119.4	1.6	1.6
MUSSELS	12	56.5	135.8	0.8	1.5
OYSTERS	2	40.0	60.0	0.5	0.8
SCALLOPS	6	82.4	167.2	1.0	1.8

As can be seen from Table 8, average portion size ranged from 40-119.4 g and the maximum portion size ranged from 60-167.2 g across the four types of molluscs consumed. In total, 21 eating occasions were recorded by a total of 16 consumers, which represents 1.07% of the entire survey population (n = 1,500) and reflects the general low consumption of shellfish in Ireland. The average and maximum portion size for each mollusc type, expressed on a g/kg bw basis was used to estimate exposure of the adult population in Ireland to the toxin groups listed in Table 7. As only one single eating occasion was recorded for cockles, this shellfish was excluded from the exposure calculations.

Potential exposure was also calculated based on the portion size of 400 g mussels, which is used by EFSA for risk assessment purposes (EFSA, 2009). The EFSA portion size of 400 g has been used also to estimate exposure from bivalves other than mussels and has been converted into g/kg bw according to the bodyweight of the Irish consumers of these products, as recorded in the NANS (see Table 9).

Table 9. Average and maximum portion sizes of bivalve molluscs expressed in grams/eating occasion and grams/kg bw/eating occasion as reported by EFSA

Bivalve Mollusc	EFSA Portion (g/eating occasion)	EFSA Portion (g/kg bw/eating occasion)**	
		Average	Maximum
COCKLES	400*	5.3	5.3
MUSSELS	400	5.7	7.8
OYSTERS	400*	5.1	5.5
SCALLOPS	400*	5.1	6.4

* The portion size reported by EFSA refers to mussels only, and has been extrapolated to other bivalve molluscs assessed in this report

** Differences observed are due to weight differences in Irish consumers of these products

The portion sizes derived for Irish consumers shown in Table 8, which were converted into raw weight equivalents to correct for potential weight loss during cooking, and the EFSA portion sizes shown in Table 9 were combined with the permitted MLs for each toxin, to estimate theoretical average and maximum exposure to marine biotoxins for each type of bivalve mollusc (Table 10). All exposure estimates were calculated on a kg bw basis to facilitate comparison with the health-based guidance value (HBGV), the ARfD, which is also expressed on a kg bw basis. This comparison was undertaken to examine if potential exposure at the MLs based on the typical Irish consumption pattern would exceed the ARfDs. Based on an evaluation undertaken by EFSA in 2009, it is known that regulatory limits are not sufficiently protective when based on the 400 g portion size (EFSA, 2009d). Therefore, exposure estimates based on the latter have been incorporated into Table 10 for comparative reasons only.

Table 10. Theoretical regulatory maximum level exposure to marine biotoxins (µg/kg bodyweight) (a) based on average and maximum Irish portion sizes derived from NANS and (b) based on the EFSA portion size, combined with maximum permitted legislative levels

	Exposure based on Irish portion sizes				Exposure based on EFSA portion size			
PSP (ML 0.8 µg/g; ARfD 0.5 µg STX equivalent/kg bw)								
	Average portion exposure		Maximum portion exposure		Average portion exposure		Maximum portion exposure	
	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD
MUSSELS	0.6	122%	1.2	241%	4.5	907%	6.2	1,248%
OYSTERS	12	56.5%	135.8	0.8%	4.1	819%	4.4	876%
SCALLOPS	2	40.0%	60.0	0.5%	4.1	813%	5.2	1,031%
DSP (ML 0.16 µg/g; ARfD 0.3 µg OA equivalent/kg bw)								
	Average portion exposure		Maximum portion exposure		Average portion exposure		Maximum portion exposure	
	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD
MUSSELS	0.12	41%	0.24	80%	0.91	302%	1.25	416%
OYSTERS (*C.gigas*)	0.08	28%	0.13	44%	0.82	273%	0.88	292%
OYSTERS (*O.edulis*)	0.08	28%	0.13	44%	0.82	273%	0.88	292%
SCALLOPS	0.16	53%	0.28	95%	0.81	271%	1.03	344%
DSP (ML 0.16 µg/g; ARfD 0.8 µg PTX2 equivalent/kg bw)								
	Average portion exposure		Maximum portion exposure		Average portion exposure		Maximum portion exposure	
	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD
MUSSELS	0.12	15%	0.24	30%	0.91	113%	1.25	156%
OYSTERS (*C.gigas*)	0.08	11%	0.13	16%	0.82	102%	0.88	109%
OYSTERS (*O.edulis*)	0.08	11%	0.13	16%	0.82	102%	0.88	109%
SCALLOPS	0.16	20%	0.28	36%	0.81	102%	1.03	129%
ASP (ML 20 µg/g; ARfD 30 µg DA+epi-DA/kg bw)								
	Average portion exposure		Maximum portion exposure		Average portion exposure		Maximum portion exposure	
	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD
MUSSELS	15.3	51%	30.1	100%	113.4	378%	155.9	520%
OYSTERS	10.6	35%	16.4	55%	102.3	341%	109.4	365%
SCALLOPS	20.0	67%	35.5	118%	101.6	339%	128.9	430%
AZP (ML 0.16 µg/g; ARfD 0.2 µg AZA1 equivalent/kg bw)								
	Average portion exposure		Maximum portion exposure		Average portion exposure		Maximum portion exposure	
	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD	µg/kg bw	% ARfD
MUSSELS	0.12	61%	0.24	121%	0.91	453%	1.25	624%
OYSTERS (*C.gigas*)	0.08	42%	0.13	66%	0.82	409%	0.88	438%
OYSTERS (*O.edulis*)	0.08	42%	0.13	66%	0.82	409%	0.88	438%
SCALLOPS	0.16	80%	0.28	142%	0.81	406%	1.03	516%

Table 10 provides the exposure estimates based on the theoretical worst case scenario assumption that the shellfish biotoxins are present at the maximum permitted level. These estimates show that exposure at the MLs based on typical Irish consumption patterns could lead to exceedance of the respective ARfDs in several cases.

6.4.1 PSP

Apart from exposure to PSP toxin from an average portion of oysters, dietary intake estimates derived for PSP toxin all exceeded the ARfD.

The EFSA Expert Panel on Contaminants (CONTAM Panel) established an ARfD of 0.5 µg STX equivalents/kg bw for PSP toxin, applying an uncertainty factor of 3 to extrapolate from the lowest-observed-adverse-effect-level (LOAEL) of 1.5 µg/kg bw to a no-observed-adverse-effect level (NOAEL) of 0.5 µg STX equivalents/kg bw. No additional factor for variation among humans was deemed necessary because the data covered a large number of affected consumers, including sensitive individuals (EFSA, 2009).

The highest theoretical level of exposure to PSP toxin was observed following consumption of a maximum portion of scallops, leading to potential intake of 1.4 µg/kg bw of PSP toxin. This value is very close to the LOAEL of 1.5 µg STX equivalents/kg bw, identified by EFSA. Therefore, at the observed maximum theoretical exposure, the possibility of onset of adverse effects in individuals sensitive to PSP toxins cannot be excluded. However, the risk of contracting PSP from shellfish produced in Ireland is considered to be very low, due to the limited occurrence of PSP toxins in Ireland, which is monitored very carefully. In addition, phytoplankton testing as an early indicator of potential PSP toxin occurrence is carried out on a continuous basis.

6.4.2 DSP

Dietary intake estimates were below the ARfDs for DSP toxins (0.3 µg OA eq./kg bw and 0.8 µg PTX2 eq./kg bw) for both average and large portion size consumers.

6.4.3 ASP

Apart from exposure to ASP toxin from consumption of a maximum portion of mussels and scallops, dietary intake estimates derived for ASP toxin were below the ARfD.

Compared to PSP, the potential exceedances of the ARfD are relatively minor, with maximum portion consumption of mussels and scallops potentially leading to intakes of 30 µg/kg bw (100% ARfD) and 36 µg/kg bw (118% ARfD), respectively.

The EFSA CONTAM Panel established an ARfD of 30 µg DA/kg bw by applying an overall uncertainty factor of 30 to the LOAEL of 0.9 mg/kg bw associated with mild signs and symptoms. The overall uncertainty factor of 30 represents a factor of 3 for extrapolation from a LOAEL to a no-observed-adverse-effect level (NOAEL), and a factor of 10 to allow for human variability and also for the fact that sensitive methods for the detection of neurotoxic effects had not been used in the investigation of affected individuals. Severe and irreversible effects were associated with exposure to DA at about 4 mg/kg bodyweight in humans (nine individuals) based on few data from ASP outbreaks (EFSA, 2009). The estimated exposure values derived following the worst case scenario in this study are considerably (~30 fold) lower than the reported LOAEL, and the risk of developing adverse symptoms is therefore deemed low.

6.4.3 AZP

Apart from exposure to AZP toxin from a maximum portion of mussels and scallops, dietary intake estimates derived for AZP toxin were below the ARfD.

The EFSA CONTAM Panel established an ARfD of 0.2 µg AZA1 equivalents/kg bw by applying an overall uncertainty factor of 9 to the LOAEL of 1.9 µg AZA1 equivalents/kg bw associated with mild signs and symptoms. The overall uncertainty factor of 9 represents a factor of 3 for extrapolation from a LOAEL to a NOAEL, and a factor of 3 because the available data related to a small number of individuals from a single incident (EFSA, 2008a). No additional factor for variation among humans was deemed necessary because the underlying data were derived from an incident showing mild and reversible effects in sensitive individuals.

The highest theoretical level of exposure to AZA toxin was observed following consumption of a maximum portion of scallops, leading to potential intake of 0.28 µg/kg bw of AZA toxin. This value was above the ARfD but below the NOAEL of 0.63 µg AZA1 equivalents/kg bw, thereby eroding part of the uncertainty factor built into the ARfD. As the NOAEL was derived from an incident involving sensitive individuals and is based on mild and reversible effects, the risk of developing adverse symptoms is therefore, considered to be low.

6.5 Uncertainty Analysis

The assessment was carried out with a view to providing a theoretical regulatory maximum level exposure assessment scenario for the adult population resident in Ireland. It assumes that the shellfish toxins of interest are present at the maximum limit, which is a worst case assumption. Shellfish reaching the market typically contain non-detectable or much lower concentrations of these toxins, and while accumulation of all modelled toxins in Irish shellfish is theoretically possible, in practice this is not the case. Regulation 853/2004 specifies that shellfish "*must not contain marine biotoxins in total quantities (measured in the whole body or any part edible separately)*" exceeding the maximum limits as specified in that legislation. For the purposes of the exposure assessment carried out in this report, the assumption was made that the maximum limits would be reached in the edible tissue, which is an appropriate assumption for oysters and mussels, where the entire animal is being consumed. This assumption, however, is overly conservative for scallops, because the part of the animal, i.e. hepatopancreas, in which the biotoxins accumulate, is removed prior to consumption.

Estimated maximum theoretical exposure carried out was based on portion sizes recorded in the NANS Irish adult food consumption survey however, only a limited number of subjects reported intake of bivalve molluscs. In total, 21 eating occasions were recorded by a total of 16 consumers, which represents 1.07% of the entire survey population. Consequently, the information on portion sizes derived from this very limited number of subjects is surrounded by a considerable amount of uncertainty and can only be regarded as indicative.

The potential change in toxin concentrations due to loss of water during cooking has been taken into account in this exposure assessment however, the factors assigned may both over and/or underestimate the true change in weight of the shellfish during preparation.

Any potential increases in toxin concentrations due to tissue re-distribution or biotransformation from one toxin analogue into another has not been taken into account and may lead to an underestimation of exposure when shellfish are consumed after cooking, as opposed to raw.

CHAPTER 7. DISCUSSION

The monitoring of shellfish produce for the presence of marine biotoxins is essential to reduce the risk to the consumers of Irish shellfish. Due to the complexities associated with marine biotoxin formation, regulators face many challenges. In general, the existing monitoring programmes and regulation of production in shellfish producing areas provides adequate protection against outbreaks of shellfish toxicity among consumers of Irish shellfish. The continuing success of this programme depends upon ongoing research to identify any changes in the geographical or temporal distribution of existing HABs or the introduction of novel toxin producing HABs into Irish shellfish growing waters.

The dynamics driving HABs are complex. Factors influencing an outbreak in one localised region may not have an impact in another and algal blooms can occur in very confined marine locations. Detailed knowledge of their ecological features and in particular, their hydrographical features, is required. The potentially local nature of HABs, combined with the range of different shellfish environments, can lead to large variation in shellfish exposure and toxin uptake. A review of PSP toxins in bivalve molluscs carried out by Bricelj and Shumway (1998) revealed a substantial range in shellfish toxicity that can develop due to non-uniform distribution of algal cells.

Concurrent phytoplankton monitoring may help to reduce the risks by indicating when toxin levels will change, e.g. during algal blooms, and thus, which shellfish will require further monitoring. However, this approach does not work equally well across all biotoxins of interest as there is not always a correlation between phytoplankton levels in water and toxin concentration in shellfish, e.g. when *Alexandrium* species cell counts increase, samples are tested for the presence of PSP. However, the presence of *Alexandrium*, even at high cell counts per litre, does not guarantee PSP production (as seen in 2011). Recent monitoring of ASP outbreaks has shown that time of year of algal blooms has an impact, and monitoring of phytoplankton counts alone may not be sufficient. Monitoring *Pseudo-nitzschia* levels in indicator species may indicate when a bloom is imminent, e.g. mussels have both a fast uptake and elimination of DA (Schwacke *et al.*, 2013; Botana, Luis M, 2014), and may provide early warning as an indicator species for ASP and, as such, could provide an excellent addition to phytoplankton counting. There is a relationship between changing cell counts and populations of toxin producing *Dinophysis* species and DSP toxins levels. Therefore, as with ASP, an indicator species for DSP toxins would be an excellent addition to phytoplankton counting.

It is noted that the selection of an indicator species for each toxin group is complicated by different factors influencing the rates of toxin uptake and depuration. According to Lawrence *et al.* (2011), the rates of toxin uptake and depuration depends on the 'combination of species, toxin and geographic location', and they consider that the 'absence of toxicity in an indicator species is assumed to imply the absence of toxicity in other species in the growing area'. The accuracy of this conclusion should be demonstrated for each species of shellfish and for each group of toxins.

There was some indication in recent years that biotoxins typically encountered in specific areas have spread to other areas, e.g. Cork Harbour had been the only area of recurring PSP problems in shellfish, whereas County Galway showed some increase in PSP in 2013. This is suggestive of PSP spread and it is recommended that these developments be monitored very closely. Also, emergence of significant levels of tetrodotoxin in EU waters warrants research into the distribution in Irish waters.

Due to the large number of toxin groups and analogues identified thus far, and the potential for many more as technology advances, new and emerging toxins are a significant challenge for research scientists and legislators alike.

There is a dearth of legislation for a broad range of existing and emerging biotoxins and/or their analogues, often due to lack of research. Where legislation does exist, there is indication that some of the limits set are not protective of human health, based on the exposure assessment carried out as part of this study. In particular, the standard portion size used by EFSA in their risk assessments would lead to an exceedance of the respective ARfDs in all cases in the Irish context. Typical and maximum portion sizes reported for Ireland from the NANS are considerably lower than the EFSA portion size. Nonetheless, for the Irish maximum portion sizes, exceedances of the ARfDs were indicated for most toxins for some shellfish species when modelled against the legislative limits. However, these exposure estimates are theoretical, are based on a very limited amount of consumers and should be verified.

On the other hand, the regulatory limit for pectenotoxins may be too stringent, if not superfluous, as PTX has been demonstrated to only co-occur at very high levels of DSP (MI, personal communication). To date, there is no indication of human toxicity from ingestion of PTX contaminated shellfish (Munday and Reeve, 2013) and further research studies are required to support de-regulation of PTX.

There is a general lack of long-term toxicity data for marine biotoxins, and although genotoxicity testing has been carried out, no chronic toxicity or carcinogenicity studies or evaluation of the potential reproductive and developmental toxicity by use of standard tests have been reported (Munday and Reeve, 2013). As a consequence, no HBGVs for chronic exposure have been set.

Ireland has a successful monitoring system which can provide predictions of toxin increases and a limited forecasting, but faces many challenges due to the high number of factors and variables involved. As has been shown by the results presented for 2011-2013, the rigorous monitoring programme in place in Ireland has prevented produce with high levels of biotoxins reaching the consumer. Potential exposure to such levels would lead to substantial exceedances of the ARfDs; this highlights the importance of regular testing and imposing closures immediately when toxins levels are above the regulatory limits.

CHAPTER 8. RECOMMENDATIONS

Ireland has a successful monitoring system which can provide predictions of toxin increases and a limited forecasting, but faces many challenges due to the high number of factors and variables involved. The Committee recommends the implementation of the following measures to further strengthen the existing monitoring and control systems.

Research

1. In production areas where more than one species of shellfish is produced, the most susceptible shellfish may be chosen as an indicator species. Assessments to identify appropriate indicator species for Amnesic Shellfish Poisoning, Diarrhetic Shellfish Poisoning, Paralytic Shellfish Poisoning and Azaspiracid Shellfish Poisoning toxins should be carried out.
2. The potential impacts of a changing environment (via climate change or ballast water) leading to range expansion or alien introduction of non-native Harmful Algal Blooms should be assessed.
3. Because of the general lack of chronic and sub-chronic toxicity data for shellfish toxins, further studies are warranted.
4. The need for harmonisation of sample pre-treatment practices, i.e. cooking versus non-cooking, before the actual analysis of lipophilic marine biotoxins, as recommended by EFSA (EFSA, 2009e), should be further explored.
5. A targeted survey of shellfish consumption in Ireland should be undertaken to verify the exposure to shellfish toxins derived in this report, which was conducted on a very limited number of consumers. Such a survey would also further support the indication that portion sizes in Ireland are considerably lower than the large portion size of 400 g used by EFSA (EFSA, 2009).

Regulation

6. Continued investigation into the occurrence and toxicity of azaspiracid analogues other than azaspiracid 1-3, is essential and the legislation needs to be amended to include them, where warranted.
7. There is a need for toxicity and epidemiological data on pectenotoxins to evaluate the proposed de-regulation of Pectenotoxins.
8. Long-term data for new and emerging toxins should be collected and regulations (and mitigation strategies) put in place to protect consumers, where warranted.

Monitoring

9. Harmful Algal Bloom development should be monitored on a local, individual production area scale to establish long term baseline data as part of ongoing official controls.
10. Levels of Paralytic Shellfish Poisoning toxins in areas where previously not encountered, e.g. Galway, should be closely monitored.
11. Monitoring and control of imported fish is recommended for certain biotoxins, such as Tetrodotoxin and Ciguatoxins.

REFERENCES

Atlantic Area Transnational Programme (2009) Advanced Tests about New Toxins appeared in Atlantic Area (online), available: http://atlanticprojects.ccdr- n.pt/project-area/atlantox/project_view?page=Project%20details [accessed 01 July 2014]

Boalch, GT, Harbour, DS (1977) Unusual diatom off the coast of south-west England and its effect on fishing. *Nature* 269, pp 687-688

Bord Iascaigh Mhara (2013) BIM Annual Aquaculture Survey

Bord Iascaigh Mhara (2014) BIM Annual Aquaculture Survey

Botana, Luis M (Ed.) (2014) 'Legal Regulations for toxins and detection methods' Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection, Third. ed. CRC Press, United Kingdom, page 46, image

Botana, Luis M (Ed.) (2014) 'Parameters that should be controlled in a monitoring program', Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection, Third. ed. CRC Press, United Kingdom, page 43, image

Botana, Luis M (Ed.) (2014) Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection, Third. ed. CRC Press, United Kingdom

Botana, Luis M (Ed.) (2014) Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection, Third. ed. CRC Press, United Kingdom. Pp143-147

Botana, Luis M (Ed.) (2014) Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection, Third. ed. CRC Press, United Kingdom. P 748

Boyce, D. G., Lewis, M. R. & Worm, B. (2010) Global phytoplankton decline over the past century. *Nature* 466, 591–596

Bresnan E *et al.* (2013) Impacts of climate change on Harmful Algal Blooms. *Marine Climate Change Impacts Partnership*: Science Review, 4(1), pp 1-8

Bricelj, VM, Shumway, SE (1998) 'Paralytic shellfish toxins in bivalve molluscs: Occurrence, transfer kinetics, and biotransformation'. *Reviews in Fisheries Science* 6(4), pp 315-383

Callaway, R. *et al.* (2012) Review of Climate Impacts on Marine Aquaculture in the UK and Ireland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, Volume 22, pp 389-421

Cangelosi, AA, Mays, NL, Balcer, MD, Reavie, ED, Reid, DM, Sturtevant, R, Gao, X, (2007) The response of zooplankton and phytoplankton from the North American Great Lakes to filtration. *Harmful Algae* 6, pp 547-566

Carmicheal, WW, (2001) Health effects of toxin-producing dyano-bacteria: "The CyanoHABs". Human and Ecological Risk Assessment. *Hum. Ecol. Risk Assess.* 7(5), pp1393-1407

Code of Practice for the Irish Shellfish Monitoring Programme (Biotoxins) (2013) Marine Institute

Codex Alimentarius International Food Standards (2014) Safe, good food for everyone [online], available: http://www.codexalimentarius.org/ [accessed 15 July 2014]

Cosgrove, S., Rathaille, A.N., Raine, R., (2014) The influence of bloom intensity on the encystment rate and persistence of *Alexandrium minutum* in Cork Harbour, Ireland. *Harmful Algae* 31, pp 114–124. doi:10.1016/j.hal.2013.10.015

Cosper, EM, Dennison, WC, Carpenter, EJ, Bricelj, VM, Mitchell, JG, Juenstner, SH, Colflesh, D, Dewey, M, (1987) Recurrent and persistent brown tide blooms perturb coastal marine ecosystem. *Estuaries* 10, pp 284-290

Doney, S.C., Balch, W.M., Fabry, V.J., Feely, R.A., (2009) Ocean acidification: a critical emerging problem for the ocean sciences.

Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., (2009) Ocean Acidification: The Other CO 2 Problem. *Annual Review of Marine Science* 1, 169–192 doi:10.1146/annurev.marine.010908.163834

Dörr, B. V. (2014) Investigation of the genotoxic potential of the marine biotoxins okadaic acid and azaspiracids. PhD Thesis Retrieved from https://cora.ucc.ie/handle/10468/1788

EFSA (2008a) Marine biotoxins in shellfish – Azaspiracid group Scientific Opinion of the Panel on Contaminants in the Food chain. *EFSA Journal* (2008) 723, 1-52

EFSA (2008b) Marine biotoxins in shellfish – okadaic acid and analogues. *EFSA Journal* 589, pp 1-62

EFSA (2008c) Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish – yessotoxin group. *EFSA Journal* 907, pp 1-62

EFSA (2009a) Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish - pectenotoxin group. *EFSA Journal* 1109, pp 1-47

EFSA (2009b) Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish – saxitoxin group. *EFSA Journal* 1109, pp 1-76

EFSA (2009c) Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish – domoic acid. *EFSA Journal* 1181, pp 1-61

EFSA (2009d) Marine biotoxins in shellfish – Summary on Regulated Marine Biotoxins Scientific. *EFSA Journal* (2009) 1306, 1-23

EFSA (2009e) Influence of processing on the levels of lipophilic marine biotoxins in bivalve molluscs EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA Journal* (2009) 1016, 1-10

EFSA (2010a) Scientific opinion on marine biotoxins in shellfish – Emerging toxins: the brevetoxin group. *EFSA Journal* 8(7) 1677

EFSA (2010b) Scientific Opinion on marine biotoxins in shellfish – Cyclic imines (spirolides, gymnodimines, pinnatoxins and pteriatoxins). *EFSA Journal* 8(6) 1628

European Communities Official Journal L 075, 16/03/2002, 2002, 0064

Friedman, MA, Fleming, LE, Fernandez, M *et al*. (2008) Ciguatera fish poisoning: Treatment, prevention and management. *Mar Drugs* (Special Issue on Marine Toxins) 6: pp456-479

Food Safety Authority of Ireland (2014) Code of Practice for the Irish Shellfish Monitoring Programme (Biotoxins) Version 5 December 2014. Retrieved February 15 2015 from: www.fsai.ie/uploadedFiles/about_Us/Industry_Fora/MSSC/CoP_Biotoxin_Monitoring.pdf

Frost, M. *et al*. (2012) Impacts of climate change on fish, fisheries and aquaculture. *Aquatic Conservation: Marine and Freshwater Ecosystems*, Volume 2(6), pp. 1251-1259

Fujiki H, Suganuma M, Suguri H, Yoshizawa S, Takagi K, Uda N, Wakamatsu K, Yamada K, Murata M, Yasumoto T. (1988) Diarrhetic shellfish toxin, dinophysistoxin-1, is a potent tumor promoter on mouse skin. *Jpn J Cancer Res*, 79(10):1089–1093

Giacobbe, M.G., Penna, A., Gangemi, E., Maso, M., Garces, E., Fraga, S., Bravo, I., Azzaro, F., Penna, N. (2007) Recurrent high-biomass blooms of *Alexandrium* taylorii (Dinophyceae), a HAB species expanding in the Mediterranean. *Hydrobiologia* 580 (1) 125–133

Gosling, E. (2003) Bivalve Molluscs. Blackwell Publishing, Oxford, UK

Gregg, MD, Hallegraeff, GM. (2007) Efficacy of three commercially available ballast water biocides against vegetative microalgae, dinoflagellate cysts and bacteria. *Harmful Algae* 6, pp 567-584.

Hallegraeff, GM. (1998) Transport of toxic dinoflagellates via ships' ballast water: bio- economic risk assessment and efficacy of possible ballast water management strategies. *Mar. Ecol. Prog. Ser.* 168, pp 297-309

Hallegraeff, GM, Steffensen, DA, Wetherbee, R. (1988) Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. *J. Plankton Res.* 10, pp 533-541.

Health Promotion Unit, Department of Health and Children, Use the Food Pyramid to plan your healthy food choices every day and watch your portion size [online], available: http://www.irishheart.ie/media/pub/charts/foodpyramid.pdf [accessed 20 July 2014]

Hibbeln, J.R., Davis, J.M., Steer, C., Emmett, P., Rogers, I., Williams, C., Golding, J. (2007) Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet* 369, 578 -585

Kilcoyne, J., McCarron P., Hess P., and Miles C.O. (2015) Effects of Heating on Proportions of Azaspiracids 1–10 in Mussels (*Mytilus edulis*) and Identification of Carboxylated Precursors for Azaspiracid-5, -10, -13 and -15, *Journal of Agriculture and Food Chemistry* (Submitted)

Kiernan M.C., Isbister G.K., Lin C.S., Burke D., Bostock H. (2005) Acute tetrodotoxin-induced neurotoxicity after ingestion of puffer fish. *Ann Neurol*. 2005 Mar;57(3):339-48

Ishige M, Satoh N, Yasumoto T. (1988) Pathological studies on the mice administration with the causative agent of diarrhetic shellfish poisoning (okadaic acid and pectenotoxin-2) Hokkaidoritsu Eisei *Kenkyushoho*. 38:15–18

Joint PICES-GEOHAB-ICES Symposium. Harmful Algal Blooms and Climate Change. 19 - 22 May 2015, Gothenburg, Sweden

Lawrence, J, Loreal, H, Toyofuku, H Hess, P, Iddya, K, Ababouch, L. (2011) FAO Fisheries and Aquaculture Technical Paper 551, Assessment and Management of Biotoxin Risks in Bivalve Molluscs, Food and Agriculture Organization of the United Nations. Food and Agriculture Organization of the United Nations, Rome, Italy

McCarthy, HP, Crowder, LB. (2000) An overlooked scale of global transport: phytoplankton species richness in ships' ballast water. *Biol Invasions* 2, pp 321-322

McCarron, P.; Kilcoyne, J.; Miles, C. O.; Hess, P. (2009) Formation of azaspiracids-3, -4, -6, and -9 via decarboxylation of carboxyazaspiracid metabolites from shellfish. *J. Agric. Food Chem.*, 57, 160–169.

McCollin, T, Shanks, AM, Dunn, J. (2007) The efficiency of regional ballast water exchange: Changes in phytoplankton abundance and diversity. *Harmful Algae* 6, pp 531-546

Munday, R., Reeve, J. (2013) Risk Assessment of Shellfish *Toxins*. Toxins 5, 2109 - 2137 doi:10.3390/toxins5112109

Peperzac, L. (2003) Climate change and harmful algal blooms in the North Sea. *Acta Oecologica* Volume 24, pp S139-S144

Rodriguez P, Alfonso A, Vale C, Alfonso C, Vale P, Tellez A, *et al*. (2008) First toxicity report of tetrodotoxin and 5,6,11-trideoxyTTX in the trumpet shell Charonia lampas lampas in Europe. *Anal Chem*. 80(14):5622-9.

Scholin, CA, Gulland, F, Doucette, GJ, Benson, S, Busman, M, Chavex, FP, Cordaro, J, DeLong, R, De Vogelaere, A, Harvey, J, Haulena, M, Lefebvre, J, Lipscomb, T, Van Dolah, FM, *et al.*, (2000) Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* 403 (6765) pp 1356-1367

Schwake, LH, Gulland, FM, White, S. (2013) Sentinel Species in Oceans and Human Health, In: Laws, E. A. (Ed), *Environmental Toxicology*, Springer New York, pp 503- 528

Shumway, SE, Cembella, A. (1993) The impact of toxic algae on scallop culture and fisheries. *Rev. Fish.* Sci. 1(2), pp121-150

Smith, M.D., Roheim, C.A., Crowder, L.B., Halpern, B.S., Turnipseed, M., Anderson, J.L., Asche, F., Bourillón, L., Guttormsen, A.G., Khan, A. (2010) Sustainability and global seafood. *Science* 327, 784–786

Strohmeier, T., Strand, Ø., Cranford, P. (2009) Clearance rates of the great scallop (*Pecten maximus*) and blue mussel (*Crassostrea gigas)*) at low natural seston concentrations. *Marine Biology* 156, 1781–1795. doi:10.1007/s00227-009-1212-3

Suganuma M, Suttajit M, Suguri H, Ojika M, Yamada K, Fujika H. (1989) Specific binding of okadaic acid, a tumor promoter in mouse skin. *FEBS Lett*. 1989;250:615–618

Taylor, FJR, Fukuyo, Y and Larsen, J. (2005) 'Taxonomy of harmful dinoflagellates.' Manual on Harmful Marine Microalgae, IOC Manuals and Guides No. 33, UNESCO, France: pp 283-317

Touzet, N., Franco, J.M., & Raine, R. (2007) Influence of inorganic nutrition on growth and PSP toxin production of *Alexandrium minutum* (Dinophyceae) from Cork Harbour, Ireland. *Toxicon* Volume 50, pp 106-119

Toyofuku, H. (2006) Joint FAO/WHO/IOC activities to provide scientific advice on marine biotoxins (research report). *Marine Pollution Bulletin* 52, 1735–1745. doi:10.1016/j.marpolbul.2006.07.007

Turner AD, Powell A, Schofield A, Lees DN, Baker-Austin C. Detection of the pufferfish toxin tetrodotoxin in European bivalves, England, 2013 to 2014. *Euro Surveill*. 2015;20(2):pii=21009. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21009

van den Bergh, J.C., Nunes, P.A., Dotinga, H.M., Kooistra, W.H., Vrieling, E.G., Peperzak, L. (2002) Exotic harmful algae in marine ecosystems: an integrated biological economic–legal analysis of impacts and policies. *Marine Policy* 26, 59–74

Villegas, R., Xiang, Y.-B., Elasy, T., Li, H.-L., Yang, G., Cai, H., Ye, F., Gao, Y.-T., Shyr, Y., Zheng, W., Shu, X.-O. (2011) Fish, shellfish, and long-chain n-3 fatty acid consumption and risk of incident type 2 diabetes in middle-aged Chinese men and women. *American Journal of Clinical Nutrition* 94, 543–551. doi:10.3945/ajcn.111.013193

Watkins, S.M., Reich, A., Fleming, L.E., and Hammond, R. (2008) Neurotoxic Shellfish Poisoning. *Marine Drugs* 6, 431-455

ANNEX

Stakeholders

Food Safety Authority of Ireland (FSAI) has the statutory function of co-ordinating the enforcement of food safety legislation at national level. The principal function of the FSAI is to take all reasonable steps to ensure that food produced, distributed or marketed in the State meets the highest standards of food safety and hygiene reasonably attainable. The FSAI aims to ensure that food complies with legal requirements, or where appropriate with recognised codes of good practice. The FSAI carries out its enforcement function through service contracts with official agencies. These contracts outline an agreed level and standard of food safety activity that the agencies perform as agents of the FSAI. Both the Sea-Fisheries Protection Authority and the Marine Institute have service contracts with the FSAI.

Sea-Fisheries Protection Authority (SFPA) is responsible for the implementation and enforcement of national and EU legislation which deals with fisheries control and the health conditions for the production and placing on the market of fish, shellfish and fisheries products. The SFPA is the competent authority for the enforcement of seafood safety legislation in Ireland and operates under a service contract with the FSAI. The SFPA is responsible for food safety related controls of shellfish growing areas, transport and seafood establishments. The SFPA implements, manages and monitors the Irish Shellfish Monitoring Programme. Sea-Fisheries Protection Officers of the SFPA act as shellfish managers in shellfish production areas and monitor product traceability.

Marine Institute (MI) is the national agency responsible for marine research, technology development and innovation. It operates under a service contract with the FSAI for its food safety related responsibilities. The Institute provides essential scientific advice and a range of marine environmental monitoring services to help ensure Irish seafood products meet approved safety standards. MI is the National Reference Laboratory for the monitoring of marine biotoxins and is responsible for the analysis of both shellfish and water samples. The Institute is accredited by INAB to ISO/IEC 17025:2005. A key component of the Irish Shellfish Monitoring Programme is the Marine Institute's Harmful Algal Bloom (HABS) Database which gives easy access to up-to-date monitoring results.

Irish Shellfish Association (ISA) is the representative body which supports shellfish producers and works to ensure future sustainability and growth in the sector. The Association represents shellfish producers' interests at local, National and European levels on issues that impact on them such as biotoxins, licensing and food safety regulation. Shellfish producers have primary responsibility for ensuring the safety of the food they produce and as such their active support and co-operation is key to the success of the Irish Shellfish Monitoring Programme. Producers actively support the programme through their work as shellfish samplers.

Food Consumption Data

National Adult Nutrition Survey (NANS) (IUNA, 2011)

Table 11. Bivalve mollusc eating occasions extracted from the NANS database (adults resident in Ireland > 18 years)

Gender	Age Group	Body Weight kg	Meal Type	Location	Quanti-fication	Category	Food consumed	Cooking Method	Food Weight kg	Ingredient	Ingredient Weight				EFSA portion (400g) in g/kg bw
											g	Edible portion	Raw edible portion	Raw edible portion g/kg bw	
Female	36-50	62.05	Main meal	Home	Weighed	MUSSELS	Mussels, boiled, weighed with shells	Not Cooked	75	Mussels, boiled, weighed with shells	75.0	20.3	30.2	0.49	6.45
Female	36-50	51.3	Main meal	Home	Weighed	MUSSELS	Mussels, boiled, weighed with shells	Boiled	94	Mussels, boiled, weighed with shells	94.0	25.4	37.9	0.74	7.80
Female	36-50	75.1	Main meal	Home	Weighed	MUSSELS	Mussels, boiled	Boiled	42	Mussels, boiled	42.0	42.0	62.7	0.83	5.33
Male	18-35	90.1	Main meal	Home	Weighed	MUSSELS	Mussels, boiled	Unknown	91	Mussels, boiled	91.0	91.0	135.8	1.51	4.44
Male	36-50	80.2	Main meal	Home	Weighed	MUSSELS	Seafood cocktail	Not Cooked	247	Mussels, boiled	41.0	41.0	61.2	0.76	4.99
Male	36-50	80.2	Light Meal	Home	Weighed	MUSSELS	Seafood cocktail	Not Cooked	200	Mussels, boiled	33.2	33.2	49.6	0.62	4.99
Female	36-50	77.9	Main meal	Restaurant /Hotel	Estimated	MUSSELS	Mussels, boiled	Combination	40	Mussels, boiled	40.0	40.0	59.7	0.77	5.13
Female	36-50	57.2	Main Meal	Restaurant /Hotel	Estimated	MUSSELS	Mussels, boiled	Unknown	28	Mussels, boiled	28.0	28.0	41.8	0.73	6.99
Male	>65	74.65	Main meal	Restaurant /Hotel	Weighed	MUSSELS	Mussels, boiled	Boiled	20	Mussels, boiled	20.0	20.0	29.9	0.40	5.36
Male	>65	74.65	Light Meal	Home	Weighed	MUSSELS	Mussels, boiled	Boiled	20	Mussels, boiled	20.0	20.0	29.9	0.40	5.36
Female	36-50	70.4	Main meal	Home	Weighed	MUSSELS	Paella	Boiled	325	Mussels, raw	33.0	33.0	34.7	0.49	5.68
Female	36-50	72.55	Main meal	Home	Estimated	MUSSELS	Mussels, boiled	Boiled	70	Mussels, boiled	70.0	70.0	104.5	1.44	5.51
Male	>65	73.1	Light Meal	Home	Food Portion Sizes (HC)	OYSTERS	Oysters, raw	Not Cooked	60	Oysters, raw	60.0	60.0	60.0	0.82	5.47
Male	36-50	84	Main meal	Restaurant /Hotel	Household Measure	OYSTERS	Oysters, raw	Unknown	20	Oysters, raw	20.0	20.0	20.0	0.24	4.76
Male	51-64	94.05	Main meal	Friend's Home	Weighed	SCALLOPS	Scallops, steamed	Unknown	112	Scallops, steamed	112.0	112.0	167.2	1.78	4.25
Female	36-50	62.05	Main meal	Home	Weighed	SCALLOPS	Scallops, steamed	Fried/stir-fried	35	Scallops, steamed	35.0	35.0	52.2	0.84	6.45
Female	18-35	83	Main meal	Restaurant /Hotel	Estimated	SCALLOPS	Scallops, steamed	Not Cooked	80	Scallops, steamed	80.0	80.0	119.4	1.44	4.82
Male	36-50	80.2	Main meal	Home	Weighed	SCALLOPS	Seafood cocktail	Not Cooked	247	Scallops, steamed	41.0	41.0	61.2	0.76	4.99
Male	36-50	80.2	Light Meal	Home	Weighed	SCALLOPS	Seafood cocktail	Not Cooked	200	Scallops, steamed	33.2	33.2	49.6	0.62	4.99
Female	18-35	80.3	Main meal	Restaurant /Hotel	Estimated	SCALLOPS	Scallops, steamed	Unknown	30	Scallops, steamed	30.0	30.0	44.8	0.56	4.98
Male	>65	75*	Light Meal	Home	Food Atlas	COCKLES	Cockles, boiled	Boiled	80	Cockles, boiled	80.0	80.0	119.4	1.59	5.33

ACKNOWLEDGEMENTS

Scientific Committee

Prof. Albert Flynn (Chair)
University College, Cork

Dr Dan O'Sullivan
Pesticide Control Service, Department of Agriculture, Food and the Marine (retired and resigned from committee 2014)

Dr Colette Bonner
Department of Health

Prof Martin Cormican
University College Hospital, Galway

Dr Geraldine Duffy
Teagasc

Prof Peter Jones
University College, Cork

Prof. Brian McKenna
University College, Dublin

Dr Paul McKeown
Health Protection Surveillance Centre

Dr Michael O'Keeffe
Residue Specialist

Dr Margaret B. O'Sullivan
Health Service Executive

Mr Ray Parle
Health Service Executive

Ms Ita Saul
Our Lady's Children's Hospital, Crumlin

Dr Paula Barry Walsh
Department of Agriculture, Food and the Marine

Technical Secretariat to the Scientific Committee

Dr Wayne Anderson
Food Safety Authority of Ireland

Secretariat to the Scientific Committee

Ms Eileen Lippert
Food Safety Authority of Ireland

Chemical Safety Sub-Committee

Dr Michael O'Keeffe (Chair)
Residue Specialist

Dr Dan O'Sullivan
Dept of Agriculture, Food and the Marine (Deputy Chair; resigned Dec 2014)

Dr Thomasina Barron
Department of Agriculture, Food and the Marine

Dr Padraig Burke
Health Service Executive

Dr Claire Chambers
Chambers Toxicological Consulting

Dr Colman Concannon
Environmental Protection Agency (Deputy Chair from Jan 2015)

Ms Catherine Cosgrove
Health Service Executive

Dr Martin Danaher
Teagasc

Dr John Keegan
Health Service Executive

Dr Peadar Lawlor
Teagasc

Mr Gerry Lohan
Department of Agriculture, Food and the Marine

Dr Evin McGovern
Marine Institute

Dr David McGrath
Retired, Teagasc

Dr John Moriarty
Department of Agriculture, Food and the Marine

Mr Joe Silke
Marine Institute

Technical Secretariat to the Chemical Safety Sub-Committee

Dr Christina Tlustos
Food Safety Authority of Ireland

Secretariat to the Chemical Safety Sub-Committee

Ms Eileen Lippert
Food Safety Authority of Ireland

Food Safety Authority of Ireland
Abbey Court, Lower Abbey Street,
Dublin 1. DO1 W2H4

Udarás Sábháilteachta Bia na hEireann
Cúirt na Mainistreach, Sráid na Mainistrach íocht.,
Baile Átha Cliath 1. DO1 W2H4

Telephone: +353 1 817 1300
E-mail: info@fsai.ie
www.fsai.ie