# FINAL REPORT

# A critical review of the current evidence for the use of indicator shellfish species for the purposes of biotoxin and chemical contaminants monitoring in the Scottish shellfish production areas

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# SUMMARY

Scotland has a diverse bivalve shellfish industry, with around 160 active in-shore production areas, which are commercially classified for a range of bivalve species. Bivalve shellfish are filter-feeding organisms and algal blooms can lead to high levels of toxins being accumulated by shellfish through feeding. Other chemical contaminants (e.g. metals and organic compounds) are also accumulated by shellfish via filter feeding or direct absorption by the gills. To protect human health, statutory monitoring of a range of toxins and contaminants in shellfish is undertaken as part of the official control programme in Scotland. To conserve resources, often one species (the so called "indicator") within an area is monitored for marine toxins and provides an indication of risk for other shellfish species produced in the same location. In Scotland, mussels are the most commonly used indicator species. Cockles, Pacific oysters and razors are also used as indicator species in a few areas. Indicator species are not currently used to support food safety risk management of contaminants in shellfish in Scotland. To assist in evaluating the evidence base for the use of particular shellfish species as indicators, a review of the literature has been undertaken, including collation of information on the worldwide uptake of indicator species in shellfish management programmes and an analysis of comparative toxin accumulation by different bivalves using historical marine toxin monitoring data generated in Scotland to date.

The review revealed interspecies differences in toxin accumulation, and confirmed the widely held belief that mussels are highly efficient accumulators of marine toxins and generally accumulate higher concentrations than most other bivalve species during algal blooms. This was supported by data generated through concurrent monitoring of multiple species in the official control (OC) programme in Scotland (2010 – 2013), which revealed 10 occasions on which toxins (paralytic shellfish poisons (PSP), diarrhetic shellfish poisons (DSP) and yessotoxins) were detected in mussels in the absence of toxicity in other shellfish species. However, while the findings of the literature review and data analysis support the general utility of mussels as an indicator species, some exceptions of relevance to Scotland were noted:

- Pacific oysters can accumulate significant quantities of azaspiracids (AZA), and at times have been reported to contain higher concentrations than mussels co-sampled from the same area at the same time.
- Studies suggest that King scallops can accumulate higher levels of amnesic shellfish poisons (ASP) and PSP than co-occurring mussels. Data from Scotland's OC programme shows three occasions on which scallops contained elevated concentrations of ASP in the gonad and/or whole tissue, while mussels (co-sampled) did not contain detectable levels.

With respect to toxin elimination, the literature demonstrates that depuration rates of toxins differ between bivalve species. Various examples were noted in which certain toxins were eliminated at slower rates from cockles, scallops, oysters and clams, when compared to mussels. This provides some explanation as to why most countries surveyed as part of this review test each species individually to facilitate re-opening of production areas following toxin bloom events, rather than relying on the results from an indicator species such as mussels.

Regarding the regulated chemical contaminants, the published literature suggests that oysters accumulate significantly higher concentrations of cadmium than mussels. Similarly, there is a small amount of evidence that polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) may be present at higher concentrations in oysters compared to mussels and cockles that co-occur in the same area. There is limited evidence

that lead and mercury may accumulate to higher levels in mussels than oysters. However, in general the evidence base for differences in accumulation of contaminants between bivalve species is weak, with few published studies undertaken which involve concurrent sampling of multiple bivalve species.

Similarly, while a significant number of publications exist on marine toxin accumulation and elimination, few studies have been specifically designed to investigate the validity of the use of indicator species for the various regulated toxin groups and commercially produced shellfish species. Thus a large number of data gaps were identified during the review process and a series of recommendations have been made regarding future research to address high priority information needs. Most of the recommendations identify a need for concurrent monitoring of particular toxins and contaminants of concern in various combinations of bivalve species: these aim to strengthen the scientific basis for policy decisions regarding which indicator species are appropriate to use in Scotland's toxin and contaminant monitoring programmes.

# EXECUTIVE SUMMARY

Scotland has a diverse bivalve shellfish industry, with around 160 active in-shore production areas which are commercially classified for a range of bivalve species including mussels, razors, Pacific oysters, cockles, surf clams, native oysters, queen scallops and king scallops. Mussels are the most significant commercial species produced in the in-shore environment[1], with around 6500 tonnes produced in 2012, valued at around £7 million.

Bivalve shellfish are filter-feeding organisms that primarily consume phytoplankton that live in the water column or on the benthos. Certain species of phytoplankton produce toxins and in some environmental conditions the plankton blooms can result in high levels of toxins being accumulated by shellfish, primarily through the process of filter feeding. Consumption of significant concentrations of some toxin types by humans can result in a variety of illnesses. Given the potential for human illness, five different groups of marine toxins are currently subjected to regulatory controls in the EU, including Scotland. The regulated toxins include: paralytic shellfish poisons (PSP), diarrhetic shellfish poisons (DSP, which includes okadaic acid, dinophysistoxins and pectenotoxins), azaspiracids (AZA), yessotoxins (YTX) and amnesic shellfish poisons (ASP).

Other chemical contaminants, such as inorganic metals and organic compounds, can also arise in the marine environment through a range of pathways that are of both anthropogenic (e.g. mining, smelting, release of wastewater etc) and biogenic (e.g. volcanic activity, natural erosion of rocks etc) origins. These contaminants can also accumulate in bivalve shellfish via direct absorption across the surface of the gills and through filter feeding on particulate matter to which the contaminants may have adhered. Particular chemical contaminants are known to cause negative health impacts in humans if long term exposure occurs, thus particular metals and organic contaminants are also subjected to regulatory controls in bivalve shellfish. Chemical contaminants that are currently regulated in bivalve shellfish include the metals cadmium (Cd), mercury (Hg) and lead (Pb), and organic compounds, which include polycyclic aromatic hydrocarbons (PAHs), dioxins and dioxin-like polychlorinated biphenyls (DL-PCBs).

To protect human health, statutory monitoring of marine toxins and chemical contaminants in shellfish is undertaken as part of the official control programme that is facilitated by the Food Standards Agency (FSA) in Scotland. Given (a) the diversity of shellfish species produced in Scotland; (b) the presence of multiple species within a single production zone; and (c) the expense associated with chemical testing programmes, often one species within an area (commonly referred to as a 'pod') is monitored for the presence of marine toxins and provides an indication of risk for all shellfish species produced in the same location. Shellfish species that are used to provide an indication of risk for other co-occurring species are frequently described as 'indicator' or 'sentinel' species. While indicator species are used for marine toxin management of shellfish, they are not currently used to support food safety risk management of contaminants in shellfish in Scotland.

Given the use of indicator species to provide information regarding the marine toxin risk of other nearby shellfish species, it is imperative that the indicator species is relatively sensitive and accumulates toxins more efficiently than other bivalve species during algal bloom

[1]A significant volume of scallops are harvested from the off-shore environment but are not included in the scope of this review due to the implementation of alternate marine toxin management procedures for 'off-shore' scallops.

events. A potential problem with indicator shellfish species is that uptake and elimination rates of contaminants may vary between bivalve shellfish, and spatial variation in occurrence of toxin producing algal blooms can occur over very small geographical scales leading to differences in exposure between species. In view of these potential issues, it is necessary to evaluate the appropriateness of particular shellfish species as indicators. To assist the FSA to evaluate the usefulness of certain indicator species, and any potential associated issues with the use of indicator species, a review of the literature has been undertaken. A summary has also been collated on the worldwide uptake of indicator species into regulatory shellfish management programmes. In addition, an analysis of comparative toxin accumulation between various bivalve species using the historical marine toxin monitoring data gathered in Scotland has been undertaken.

The main shellfish species used as an indicator organism for marine toxins in Scotland is the common mussel. Mussels are used as an indicator species for Pacific oysters (12 pods), cockles (seven pods), King scallops (two pods), native oysters (two pods) and razors (two pods). Cockles, Pacific oysters and razors are also used as indicator species in some pods. Data gathered from 12 shellfish producing countries demonstrates that most countries (11 of 12) also use indicator species in their management programmes, with all countries using mussels as the sentinel for a range of different bivalve shellfish types. The management approach taken in response to positive results in an indicator species was found to vary, with some countries restricting harvest of all species within the production area, and others increasing frequency of testing of the indicator and/or other species that may be present. Most countries (eight of 11) noted that, following the closure of production areas due to toxicity, all species present in the area were tested individually to ensure that toxin levels were below regulatory limits in all species in the area prior to re-opening.

The literature review confirmed the widely held belief that mussels are highly efficient accumulators of marine toxins and generally accumulate higher levels of toxins than most other bivalve species during algal blooms. Similarly, analysis of Scotland's marine toxin data (2010 – 2013) revealed 10 occasions on which toxins (PSP, DSP and YTX) were detected in mussels in the absence of toxicity in Pacific oysters or razors that were collected from the same location at the same time. Conversely, there were no occasions on which Pacific oysters or razors showed toxicity in the absence of toxins in mussels. Additionally, it was noted that mussels accumulated higher concentrations of toxins (with the exception of ASP) than all other species of bivalves monitored in Scotland over the period 2010 to 2013. Thus, the findings of the literature review and data analysis support the general utility of mussels as an indicator species, however there are some exceptions.

While AZAs were also generally found to accumulate to higher levels in mussels than other species, the literature base contains evidence that Pacific oysters may also concentrate significant quantities of AZA, and at times contain higher concentrations than mussels co-sampled from the same area, at the same time. However, it is unclear from the published data, whether the elevated concentrations in oysters represent a more efficient concentration process, or slower elimination rate, when compared to mussels. While there were six occasions on which Pacific oysters and mussels were concurrently sampled in Scotland and tested for AZA, none of the samples were found to contain AZA. However, between 2011 and 2013 (since the introduction of LC-MS/MS methodology to specifically identify AZA in Scotland), AZA was detected in a higher proportion of oyster samples (24.4%) than mussel samples (4.8%) collected in Scotland. This may indicate an increased propensity for oysters to accumulate and/or retain AZA as compared to mussels, or could be related to bias in the sampling strategy - the lack of concurrently collected data for oysters and mussels

sampled during AZA events prevents firm conclusions regarding comparative accumulation by these species.

Several published studies also suggest that King scallops can accumulate higher levels of ASP and PSP than co-occurring mussels. Analysis of historical monitoring data from Scotland regarding ASP levels in King scallops and mussels, which were sampled in the same location at the same time, shows three occasions on which concurrent samples were collected. On each occasion, scallops contained elevated concentrations of ASP in the gonad, whereas mussels did not contain detectable levels. On one occasion whole scallop tissue was also tested and found to have a level of 25 mg/kg ASP (compared to the maximum permissible limit of 20 mg/kg), while ASP was not detected in co-occurring mussels. Thus the literature and monitoring data suggest that King scallops have a higher propensity to accumulate ASP than mussels. Regarding PSP, there were 19 occasions in which concurrent samples of King scallops and mussels were collected and analysed, however no toxins were detected. Consequently the findings regarding PSP are inconclusive as there are no concurrent monitoring data for PSP in King scallops and mussels from Scotland during PSP-producing blooms.

There are several reasons for the apparent differences in toxin accumulation patterns between bivalve species:

1. The clearance and filtration rates of bivalves differ significantly from each other and are impacted differentially by variable temperatures and salinity. Notably, the clearance rates of scallops are considered to be relatively high when compared to other bivalve species such as mussels and oysters, perhaps contributing to the observed higher accumulation of ASP and PSP in scallops compared with mussels.
2. Variation in feeding response of bivalves to harmful algae is thought to play a significant role in the observed differences in toxin accumulation. For example, some species have the ability to modify their filtration behaviour in response to the presence of toxic algae; oysters and clams frequently exhibit valve closure and reduced clearance and filtration rates in response to the presence of harmful algae. In contrast, most studies report that scallops and mussels are largely unaffected by harmful algae and continue to feed normally, potentially accounting for the higher observed accumulation of some toxins by mussels and scallops.
3. Size of food particles plays a role in particle selection processes undertaken by bivalves, which may relate to differences in the structural arrangements of the gills of some shellfish species. It is considered that differences in uptake of algal cells of various sizes may play a role in interspecies variations in toxin accumulation.
4. A major factor that is highly likely to contribute to interspecies differences in toxin concentrations is ‘exposure’. Bivalve species preferentially inhabit different zones in the marine environment, for example scallops, clams and cockles inhabit the benthos, whereas mussels are predominantly cultured in the sub-tidal zone. Algal blooms are known to be patchy in distribution, thus shellfish located in different regions of the marine environment are likely to experience different degrees of exposure to algal toxins.

With respect to toxin elimination, the literature demonstrates that depuration rates of toxins differ significantly between bivalve species. Mussels, oysters and scallops are considered ‘fast’ detoxifiers of PSP, whereas cockles are considered to be slow detoxifiers. Consistent with this, cockles were shown to eliminate pectenotoxins at a slower rate than

mussels. The depuration rate of DSP toxins from two clam species was also found to be slower than the elimination of DSP from mussels. Several countries also noted in response to the survey distributed as part of this project, that oysters and scallops eliminate toxins (toxin type not specified) at a slower rate compared to mussels. This is consistent with the literature, which suggests that some species of scallops retain PSPs for longer periods of time than mussels. Data is, however, limited regarding the relative elimination rates of toxins between different species commercially produced in Scotland, this largely relates to a lack of concurrent data collected for multiple bivalve species during and following algal bloom events.

Regarding chemical contaminants, a reasonable body of evidence in the published literature suggests that oysters accumulate significantly higher concentrations of Cd than mussels. Similarly there is a small amount of evidence that PAHs and PCBs may be present at higher concentrations in oysters compared to mussels that co-occur in the same area. There is also limited evidence that Pb and Hg may accumulate to higher levels in mussels than oysters. However, in general the evidence base for differences in accumulation of contaminants between bivalve species is relatively weak, with few published studies undertaken which involve concurrent sampling of multiple bivalve species.

Similar to toxins, the reasons for variations in contaminant levels between bivalve species primarily relate to differences in filtration and feeding physiology. Differences in clearance rates have been associated with variations in the assimilation and absorption efficiency of metals in bivalves. Additionally, it is considered that seasonal variations impact on the concentrations of chemical contaminants in bivalves, with higher concentrations of PAHs and PCBs demonstrated in the winter months. These seasonal variations appear to be related to sexual maturity and spawning in bivalves and thus could result in interspecies differences due to variations in the time at which spawning occurs for different species (also noting that not all commercially produced bivalves naturally spawn). Similar to the situation with toxins, differences in bivalve exposure are also likely to account for interspecies variation in contaminant accumulation. This is particularly likely to be a factor with respect to bivalves that live within the sediments and may be at risk of higher exposure to sediment bound contaminants than bivalves that live in the water column. Finally, differences in contaminant concentrations between species are also related to the biological half-life of the contaminant within each bivalve species. Studies to date have demonstrated large variations in the biological half-lives of various contaminants in different bivalves. However, few studies have been undertaken to investigate the biological half-life of contaminants within different bivalve species simultaneously – this makes conclusions regarding the elimination timeframes of contaminants difficult to assess.

The review identified a range of data gaps and a series of recommendations have been made regarding future research to address high priority information needs. Key recommendations include:

1. Marine toxin data generated from concurrent sampling of indicator and representative species commonly used in Scotland are limited. In Scotland, mussels are most commonly used as an indicator species for Pacific oysters (12 pods) and cockles (seven pods). Cockles are also used as an indicator species for razors in three pods. It is recommended that when toxin-producing algal blooms occur in pods in which these particular species co-occur, that dual monitoring of these species is undertaken throughout and following the bloom events. This approach should

strengthen the evidence base for the most commonly used indicator species in Scotland.

2. To assist further appraisal of the appropriate indicator species for AZA it is recommended that concurrent monitoring of mussels and Pacific oysters is undertaken, preferably at times when AZA-producing organisms and/or AZA are known to be present in the water column.
3. To evaluate if mussels provide an adequate indication of ASP risk in King scallop meat (as opposed to gonad or hepatopancreas), it is suggested that dual sampling of scallop meat and co-located mussels is undertaken during ASP-producing *Pseudo-nitzschia* blooms.
4. It has been suggested that King scallops may be more efficient at accumulating PSP toxins than mussels, however data from Scotland's in-shore monitoring programme are limited. Consequently it is recommended that dual monitoring of scallops and mussels is undertaken during PSP bloom events.
5. In the future it may be desirable to evaluate comparative accumulation of okadaic acid (OA), dinophysistoxins (DTX) and pectenotoxins (PTX) separately – these toxins are currently reported as one value ('DSP') within the toxin database. To enable future comparisons between species for these toxins, it is recommended that the current reporting system for DSP be revised to incorporate separate fields for OA, DTX and PTX in the database, along with the current 'total DSP' field.
6. Regarding chemical contaminants, if future consideration is to be given to the introduction of an indicator approach for risk management, it is suggested that concurrent monitoring data would be required to support a science-based decision on the appropriate indicator species to use. If such an approach is taken, it is recommended that a statistically based sampling programme be designed which involves sampling multiple bivalve species at the same time from each of several different monitoring sites.
7. Currently chemical contaminant data is held in a series of Microsoft excel spread sheets. It is recommended that the historical contaminant data are collated and incorporated into a central database or spread sheet that can be used as a repository for all contaminant data generated in the future.

It is envisaged that the findings of this review will assist the FSA to meet the recent Food and Agricultural Organisation and Codex recommendations regarding the use of indicator species i.e. the need to verify that the absence of toxicity in the indicator species implies the absence of toxicity in other species within a production area. The outcomes of the study will support the future development of marine biotoxin and chemical contaminant management policies regarding the use of indicators in various production areas/pods in Scotland and enable the FSA to target future research to higher risk areas where critical data gaps exist.

# GLOSSARY

| | |
|---|---|
| α | Absorption efficiency |
| AE | Assimilation efficiency |
| Ag | Silver |
| ASP | Amnesic shellfish poisons |
| AZA | Azaspiracids |
| $b^1/_2$ | Biological half-life ($b^1/_2$) is the time (days) it takes for half of a substance to be eliminated from a mollusc |
| BAP | benzo(a)pyrene |
| Cd | Cadmium |
| CR | Clearance rate |
| DL-PCB | Dioxin like-polychlorinated biphenyls |
| DSP | Diarrhetic shellfish poisons |
| DTX | Dinophysistoxin |
| EFSA | European Food Safety Authority |
| EU | European Union |
| FAO | The United Nations Food and Agricultural Organisation |
| FR | Filtration rate |
| FSA | Food Standards Agency |
| Hg | Mercury |
| HPLC | High performance liquid chromatography |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LC-FLD | Liquid chromatography – fluorescence detection |
| LC-MS/MS | Liquid chromatography – mass spectrometry/mass spectrometry |
| LoD | Limit of detection |
| LoQ | Limit of quantitation |
| MeHg | Methylmercury |
| MPL | Maximum permissible limit |
| MT | Metallothioneins |
| MW | Molecular weight |
| PAH | Polycyclic aromatic hydrocarbon |
| Pb | Lead |
| PCB | Polychlorinated biphenyls |
| PCDD | Polychlorinated dibenzo-p-dioxin |
| PCDF | Polychlorinated dibenzofuran |
| Pod | Group of shellfish production areas that are located in close proximity to each other and thought to be hydrographically and environmentally similar |
| PSP | Paralytic shellfish poison |
| PTX | Pectenotoxin |
| RL | Reporting limit |
| RMP | Representative monitoring point |
| TCDD | tetrachlorodibenzo-*p*–dioxin |
| WHO | World Health Organisation |
| YTX | Yessotoxin |
| Zn | Zinc |

## Shellfish species names

| Scientific name | Common name |
|---|---|
| *Acanthocardia echinata* | Prickly cockle |
| *Aequipecten opercularis* | Queen scallop |
| *Anadara antiquata* | Blood cockle |
| *Argopecten irradians* | Bay scallop |
| *Artica islandica* | Icelandic cyprine |
| *Atrina vexillum* | Pen shell |
| *Aulacomya maoriana* | Ribbed mussel |
| *Austrovenus stutchburyi* | New Zealand cockle |
| *Cerastoderma edule* | Common cockle |
| *Cerastoderma glaucum* | Lagoon cockle |
| *Chama iostoma* | Rocky oyster |
| *Chlamys farreri* | Farrers scallop |
| *Chlamys nippoensis akazara* | Akazara scallop |
| *Chlamys nobilis* | Noble scallop |
| *Chlamys varia* | Varigated scallop |
| *Choromytilus meridionalis* | Black mussel |
| *Crassostrea belcheri* | Lugubrious cupped oyster |
| *Crassostrea gigas* | Pacific oysters |
| *Crassostrea iredaleii* | Slipper cupped oyster |
| *Crassostrea margaritacea* | Oyster |
| *Crassostrea rhizophorae* | Mangrove cupped oyster |
| *Crassostrea rivulans* | Suminoe oyster |
| *Crassostrea virginica* | Eastern oyster (American cupped oyster) |
| *Donax anus* | Surf clam |
| *Donax trunculus* | Abrupt wedge shell |
| *Donax vittatus* | Banded wedge shell |
| *Ensis directus* | Razor clam |
| *Ensis siliqua* and *Ensis arcuatus* | Razor clam |
| *Flexopecten proteus* | Scallop |
| *Glycymeris glycymeris* | Dog cockle |
| *Haliotis discus hannai* | Japanese abalone |
| *Holocythia roretzi* | Sea squirt |
| *Littorina littorea* | Common periwinkle |
| *Leukoma staminea* | Littleneck clam |
| *Macoma balthica* | Tiny pink clam (Baltic tellin) |
| *Macoma birmanica* | Clam |
| *Macomona liliana* | Wedge shell |
| *Mercenaria mercenaria* | Northern quahog (clam) |
| *Meretrix meretrix* | Asiatic hard clam |
| *Modiolus barbatus* | Bearded horse mussel |
| *Modiolus modiolus* | Horse mussel |
| *Musculus niger* | Black mussel |
| *Mya arenaria* | Soft shelled clam (sand gaper) |
| *Mytilus californianus* | Californian mussel |
| *Mytilus edulis* | Common mussel (blue mussel) |
| *Mytilus galloprovincialis* | Mediterranean mussel |
| *Mytilus trossulus* | Northern bay mussel |
| *Ostrea edulis* | Native oysters (European flat oyster) |

| **Scientific name** | **Common name** |
|---|---|
| *Paphies donacina* | Clam |
| *Patella vulgata* | Common limpet |
| *Patinopectin yessoensis* | Yesso scallop |
| *Pecten maximus* | King scallop (Great Atlantic scallop) |
| *Pecten novaezelandiae* | New Zealand scallop |
| *Perna canaliculus* | Greenshell mussel (New Zealand mussel) |
| *Perna perna* | South American rock mussel |
| *Perna viridis* | Green mussel |
| *Pinna nobilis* | Noble pen shell |
| *Placopecten magellanicus* | Atlantic deep sea scallop (American sea scallop) |
| *Rangia cuneata* | Clam |
| *Ruditapes decussatus* | Grooved carpet shell clam |
| *Ruditapes philippinarum* (*Tapes philippinarum*) | Manila clam (Japanese carpet shell) |
| *Saccostrea echinata* | Black lip oyster (spiny oyster) |
| *Sanguinolaria acuminata* | Clam |
| *Septifer virgatus* | Mussel |
| *Solen marginatus* | Grooved razor shell |
| *Spisula solida* | Surf clam |
| *Spisula solidissima* | Atlantic surf clam |
| *Spisula subtruncata* | Cut trough shell |
| *Spondylus squamus* | Thorny oyster |
| *Tapes semidescussatus* | Clam |
| *Venerupis senegalensis* | Pullet carpet shell |
| *Venus verrucosa* | Clam (warty venus) |

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# SECTION ONE: INTRODUCTION

## 1.1 Background

Certain species of marine phytoplankton (primarily dinoflagellates and diatoms) are known to produce toxins. Bivalve shellfish are filter feeders, and when blooms of these toxin-producing plankton occur they can accumulate high concentrations of the toxins in their digestive tracts and to a lesser degree their muscular tissues. There are a large number of different marine toxins that are produced by phytoplankton, and these are broadly categorized into eight different toxin groups (FAO, 2004; Lawrence et al., 2011). Of these eight toxin groups, the most serious risks to human health are posed by the okadaic acid toxin group, the azaspiracid toxin group, the domoic acid toxin group and the paralytic shellfish toxin group.

A variety of chemical contaminants can also arise in the marine environment from both anthropogenic and biogenic origins. Sources of contaminants include: natural erosion of rocks and sediments, degassing of the earths crust, volcanic activity, mining, smelting, release of wastewater, burning and incineration, application of fertilisers and subsequent run-off etc. Shellfish can accumulate chemical contaminants via two major pathways: (a) through filter feeding on particulate matter in which the contaminant has absorbed; and (b) through direct absorption of the contaminant across the gills. If significant quantities of certain contaminants are accumulated, people who consume the shellfish may become ill; generally illness related to the intake of chemical contaminants is caused by long-term exposure to the contaminants in the environment and/or through the regular consumption of a range of contaminated food types (i.e. acute illness is less likely).

Scotland currently has approximately 160 active in-shore shellfish production areas. Mussels (*Mytilus* spp.) are the most significant commercially produced bivalve species in Scotland by volume and value, followed by razors (*Ensis* sp.) and Pacific oysters (*Crassostrea gigas*). Small volumes of cockles (*Cerastoderma edule)*, surf clams (*Spisula solida*), native oysters (*Ostrea edulis*) and cultivated King and Queen scallops (*Pecten maximus* and *Aequipecten opercularis* respectively) are also commercially harvested from the in-shore environment. To protect human health, shellfish are monitored for a variety of toxins and contaminants that are specified in EU law (specified in Section 1.2). Given the variety of shellfish species produced in Scotland, the resource required to monitor each of these species for each of the regulated toxin and contaminant groups on a routine basis is large. Other countries face similar challenges, and a frequent approach taken both in Scotland and elsewhere is to monitor one species of shellfish in a production area to provide an indication of risk for the whole area, and for any other species of shellfish that may also be grown in it.

Ideally an indicator species (sometimes referred to as sentinel species) will provide an early warning of toxin and/or chemical contaminant presence and is relatively sensitive (compared to other bivalve species) to contaminants and toxins of concern due to its ability to rapidly accumulate or concentrate these chemical hazards. A difficulty with the use of indicator shellfish species is that toxin and contaminant uptake and elimination rates may vary between shellfish species, and spatial variation in occurrence may occur over small geographical scales creating differences in exposure between species.

In relation to this issue, the most recent advice from the United Nations Food and Agricultural Organization and the Codex Alimentarius Commission regarding the use of indicator shellfish species states the following: *"It is important to note that, using an indicator shellfish species, the absence of toxicity in the indicator species is assumed to imply*

*the absence of toxicity in other species in the growing area. This implication must be verified for each shellfish species and for each group of toxins before defining a particular shellfish species as an indicator for that growing area"* (Codex, 2009; Lawrence et al., 2011).

In order to improve knowledge and understanding regarding the use of appropriate shellfish indicator species for toxins and contaminants and any associated issues and concerns, a review of the literature has been undertaken and is described herein. A summary has also been collated on the worldwide uptake of indicator species into regulatory shellfish management programmes, the way in which indicator species are used, and any identified problems with these systems. Finally, analysis of the historical monitoring data gathered in Scotland to date, for marine biotoxins (and plankton) has been undertaken to identify circumstances in which toxicity in an indicator species was absent (or present at very low levels), when toxicity was present in other species at significant levels within the same area. Data gaps are highlighted and recommendations regarding future research to address significant data limitations are provided.

The objectives and scope of the review are discussed in more detail in Sections 1.3 and 1.4.

## 1.2 Current approach in Scotland

EC law requires a range of official controls for bivalve molluscan shellfish to ensure that shellfish contaminated with marine toxins and contaminants at concentrations exceeding maximum permitted levels are not placed on the market. The so called 'hygiene package'[2] provides regulations for the monitoring of production areas for marine toxins and chemical contaminants that may be present in shellfish. Regulation (EC) 853/2004[3] specifies maximum permissible levels for the following marine toxin groups in shellfish:

- Paralytic shellfish poisons (PSP) (synonymous with saxitoxin-group toxins and paralytic shellfish toxins)
- Amnesic shellfish poisons (ASP) (synonymous with domoic acid-group toxins)
- Diarrhetic shellfish poisons (DSP) (comprises okadaic acid, dinophysistoxins and pectenotoxins together)
- Yessotoxins (YTX)
- Azaspiracids (AZA).

Regulation (EC) 1881/2006[4] specifies the maximum levels of certain contaminants that bivalves must also comply with, including: (a) lead, cadmium and mercury; (b) polycyclic aromatic hydrocarbons (PAHs); and (c) dioxins and dioxin-like PCBs (DL-PCBs). These regulations apply to the edible parts of bivalves (notably, for King scallops the digestive gland is excluded). The current maximum permitted levels for marine toxins and chemical contaminants in bivalves are detailed in Sections 3.3.1 and 3.4.1.

Statutory routine monitoring of shellfish in Scotland is undertaken for the toxins and contaminants noted in EC law and the levels specified in the EC regulations are implemented. The frequency of shellfish testing for marine toxins is based on the outcome of risk assessments that are undertaken regularly, the last risk assessment was conducted in

[2]The hygiene package comprises the following regulations: Regulation (EC) 852/2004, Regulation (EC) 853/2004, Regulation (EC) 854/2004 and Regulation (EC) 882/2006.
[3]Regulation (EC) 853/2004 was amended by Regulation (EC) 786/2013.
[4]Regulation (EC) 1881/2006 was amended by Regulation (EC) 629/2008 (heavy metals), Regulation (EC) 835/2011 (PAHs), Regulation (EC) 1259/2011 (dioxins).

2011-2012. In addition to toxin and contaminant monitoring, identification and enumeration of potential harmful algal species is also undertaken, this complementary information can provide forewarning of marine toxin events. For chemical contaminants, the risk management programme up to 2014 involved the *ad hoc* collection of a single bivalve species (species type not mandated) from a selection of different production areas once per annum for testing of the regulated contaminants.

In relation to marine toxin management, Regulation (EC) 854/2004 has a clause that allows for the testing of indicator species in areas in which multiple species are produced, provided information is available regarding relative toxin accumulation:

*"When knowledge of toxin accumulation rates is available for a group of species growing in the same area, a species with the highest rate may be used as an indicator species. This will allow the exploitation of all species in the group if toxin levels in the indicator species are below the regulatory limits. When toxin levels in the indicator species are above the regulatory limits, harvesting of the other species is only to be allowed if further analysis on the other species shows toxin levels below the limits."*

In Scotland, 26 of the 84 management areas (commonly referred to as pods) utilise indicator bivalve species for marine toxin management purposes i.e. one species is routinely tested and the results are used to open and close the area, in which other shellfish species are also produced. It is noteworthy that in Scotland indicator species are used in pods in which multiple species are produced (19 pods), but also in some areas (seven pods) in which only one species is commercially produced but it is impractical or difficult to collect the target commercial species for toxin monitoring (e.g. mussels are used as an indicator species for two pods in which King scallops are produced on the seafloor). The Food Standards Agency note the following guidance regarding the use of indicator species in Scotland:

*"In most cases, the representative species are mussels. There is evidence from the risk assessment and from other sources that mussels are more likely to have elevated levels of toxins than other species, oysters for example. Further Regulation 854/2004 allows for the use of a representative species for the purpose of monitoring. Where mussels are not available, then the species of harvest will be the representative species"(Murray, 2009).*

Section 5 provides more detailed information on the species used as indicators and the shellfish species that the indicators represent in Scotland. Production area closures are made on the basis of toxin results obtained from the indicator species i.e. the pod is closed for all species that are grown in the area. Re-opening of production areas is also undertaken based on the results from the indicator species i.e. other species grown within the same area are not generally tested for re-opening purposes.

Regarding chemical contaminants, indicator species are not currently used for management purposes in Scotland.

## 1.3 Aim of Study

The objectives of this study are:

1. To complete a critical review of international literature on use of indicator shellfish species and relative uptake and elimination patterns for major regulated marine toxins and chemical contaminants in bivalve molluscan shellfish.

2. To compile a summary of the use of indicator shellfish species in international shellfish risk management programmes.

3. To review Scotland's toxin monitoring data to evaluate the effectiveness of indicator species and identify data gaps.

4. To develop recommendations on future research that could be undertaken to address high priority data gaps on feasibility of indicator species.

## 1.4 Scope of the Review

The scope of the review has been limited as follows:

- Examination of journal publications (primary empirical studies and secondary sources such as literature reviews), UK government documents and datasets, conference proceedings, and un-published reports;
- Synthesis of the results of studies relating to the use of shellfish indicator species, and comparative studies investigating relative accumulation and elimination of the regulated marine toxins (PSP, DSP, ASP, YTX and AZA) in multiple bivalve species collected concurrently (i.e. studies involving monitoring of two or more bivalve species at the same time and location); and
- Preference was given to reviewing literature pertaining to bivalve species that are commercially produced in Scotland (Section 3.1.3).

# SECTION TWO: METHODOLOGY

## 2.1 Literature Review

Literature searches were undertaken to collate information on:

- Factors that influence bivalve filtration and feeding;
- Use of indicator species for shellfish management;
- Toxin and contaminant localisation within shellfish; and
- Toxin and contaminant accumulation and elimination by bivalve species.

Literature searches began with a structured electronic search using the Google Scholar and PubMed search engines. Electronic literature searches commenced with the following key words:

- Bivalve, shellfish AND filtration rate, clearance rate, pumping rate
- Bivalve, shellfish AND clearance rate, filtration rate, pumping rate AND temperature, salinity
- Shellfish AND toxin, contaminant AND indicator, sentinel
- Shellfish, toxin AND accumulation, uptake
- Shellfish, toxin AND depuration, elimination
- Shellfish, toxin AND localisation, tissue distribution
- Shellfish AND Cd, Pb, Hg, PAH, dioxin, PCB AND accumulation, uptake
- Shellfish AND Cd, Pb, Hg, PAH, dioxin, PCB AND depuration, elimination
- Shellfish AND Cd, Pb, Hg, PAH, dioxin, PCB AND localisation, tissue distribution

Papers that were identified through electronic searching were assessed for relevance by initially reviewing the abstracts. Additional papers were accessed using the reference list of reviewed publications. Unpublished reports relating to the detection of toxins, contaminants and phytoplankton within Scotland were sourced by enquiry from the Food Standards Agency Scotland.

A critical appraisal of the most relevant papers included in the review was undertaken. The appraisal process used was similar to that undertaken in a recent literature review by Younger (2014). This involved the following steps:

1. Papers were initially filtered to identify those that are most relevant to the objectives of this study. The criteria used to identify papers of highest relevance included:
    - Studies in which concurrent monitoring of at least two bivalve species was undertaken at the same site;
    - Studies which involved concurrent monitoring of bivalves in the marine environment, as opposed to laboratory uptake experiments (laboratory uptake experiments were considered complementary information);
    - Studies which investigated accumulation and elimination of toxins or contaminants in bivalve species that are commercially produced in Scotland;
    - Studies involving the contaminants and toxins of regulatory concern in Scotland (other non-regulated contaminants and toxins were not considered); and
    - Studies that significantly influenced the outcomes of the literature review conclusions, as identified in Sections 3.3.5 and 3.4.5.

2. Studies of high relevance (as identified in 1 above) were evaluated using the following questions:
   - Were appropriate analytical test methodologies used for toxins and contaminants?
   - Were the different bivalve species located in the same production area, or within close proximity of each other?
   - Were samples of different species collected from the same site at the same time?
   - Was the number of field sites included in the study sufficient to support generalisations regarding relative accumulation and elimination by different bivalve species?
   - Were the number of collection events and/or samples analysed sufficient to support generalisations regarding relative accumulation and elimination by different bivalve species?
   - Did the study design, data and statistical treatment support the conclusions?

3. The questions above were evaluated for each of the high relevance papers, and a score of 0 (no), 1 (acceptable/generally) or 2 (yes) was allocated for each question. A total score was calculated for each paper, thus high scoring papers are suggestive of robust results and conclusions (a maximum score of 12 is possible). The results of the critical appraisal for each paper are presented in Appendix 1.

## 2.2 Review of monitoring data

### 2.2.1 Data sources

Data generated from shellfish monitoring undertaken as part of the official control programme for marine toxins was kindly provided by the FSA. The following documents and databases were used to support the review:

- A list of all currently classified shellfish production areas in Scotland;
- A list of the pods (management areas), commercially classified shellfish species for each pod, and the representative and alternate monitoring points and species for each pod (current at March 2014); and
- Marine toxin databases containing official control toxin data for the periods: 2001 – 2010 and October 2010 – December 2013.

Initial scrutiny of the marine toxin databases revealed several occasions in which concurrent monitoring had been undertaken, the approach used for data analysis of the toxin data is described in Sections 2.2.2 to 2.2.4.

Regarding chemical contaminants, seven data files (Microsoft excel) containing contaminant data generated between 2006 and 2013 were made available by the FSA. These data files contained heavy metal, PAH and dioxin/DL-PCB information generated from the collection of shellfish in Scotland over this time period. Initial scrutiny of these data files suggested that multiple species have not been gathered concurrently from the same location at the same time. Thus the way in which the data has been gathered prevents meaningful analysis of comparative accumulation between different species. Therefore, no formal data analysis on the chemical contaminant data was undertaken.

### 2.2.2 Sites and species analysed

Production areas are grouped into geographically close regions that are thought to be similar hydrographically and environmentally, these areas are described as pods. The number of pods and the shellfish species commercially produced in each pod was summarised. For toxin management purposes, each pod has a monitoring site from which routine samples of a particular species are collected for toxin analysis (the representative monitoring point). The species used in each pod for official control monitoring purposes was recorded. Some pods also employ an alternate monitoring species, which is monitored when the main species cannot be accessed, or there are stock limitations - this species was also recorded. Thus the bivalve species used as an indicator, and other species commercially produced in the same pod and thus represented by the indicator, were identified and summarised for each pod (Section 5). This information was used to focus the data analysis on the various combinations of indicator and representative species currently used in Scotland's official control programme.

### 2.2.3 Toxin data summaries: 2010 - 2013

To provide an overview of the prevalence and concentration of toxins in each bivalve species a series of data summaries was prepared for each regulated toxin group (e.g. PSP, DSP, AZA, YTX and ASP). The data summaries included the following information:

- The number of samples analysed for each species;
- The highest concentration of toxin recorded in each species;
- The number of detections recorded for each species;
- The number of samples that were above the maximum permissible level (MPL); and
- The number of samples in which the toxin concentration was greater than half the MPL ($^1/_2$ MPL).

For the lipophilic toxins (i.e. DSP, AZA and YTX) and PSP, three values are reported in the database, the actual value, a low value and a high value – the low and high values represent the lower and upper limits of the determined measurement uncertainty range. The high value is the current value used for regulatory purposes and was the value used in the data summaries.

For DSP, AZA and YTX, data obtained between July 2011 and December 2013 were used to prepare the summaries. This period was chosen, as this was the date when the LC-MS/MS method of analysis for these toxins was introduced in support of the official control programme in Scotland. Prior to this time these toxins were analysed using a mouse bioassay method, which provided one result for the three toxin groups and did not identify which toxin types caused positive results. The non-specificity of the DSP mouse test precludes comparisons of toxin accumulation between species, as it is unclear what toxin group caused the positive result.

For consistency a similar time period was chosen for the ASP and PSP data; data generated between October 2010 to Dec 2013 was used. Similar to the situation with DSP, this represents a period in which quantitative HPLC was routinely used for the analysis of PSP toxins, as opposed to the previously used mouse bioassay method. Lastly, it should be noted that Holtrop (2008) previously undertook an analysis of comparative accumulation of PSP and DSP by oysters and mussels. This previous analysis encompassed the period between 2001 and 2008. Thus the period chosen for this study includes data not previously analysed for between species trends.

### 2.2.4 Analysis of interspecies toxin accumulation

Initially the analysis focused on data generated between October 2010 and December 2013 for PSP and ASP, and between July 2011 and December 2013 for DSP, AZA and YTX (as noted in Section 2.2.3).

Using the information collated regarding shellfish species that are used as indicators and the species they represent in Scotland, the database was scrutinised to identify all pods in which monitoring of both the indicator and representative species co-occur. This was undertaken for the following species combinations:

- Pacific oysters and mussels
- Mussels and cockles
- Mussels and King scallops
- Mussels and native oysters
- Mussels and razors
- Cockles and razors
- Cockles and Pacific oysters
- Pacific oysters and Queen scallops
- Pacific oysters and razors

For each species pair, instances in which both species had been collected and tested from the same pod within 24 hours of each other were identified. A 24-hour period was chosen, as it is known that some species of algae can bloom rapidly with toxin levels rising significantly in a very short period (for example, AZA is documented to rise from no detectable toxicity to levels exceeding the regulatory limit within a 24 hour period (Jauffrais et al., 2012b).

It was noted that for several species pairs no concurrent monitoring data were available (Section 5). For other species pairs for which concurrent data is available, the number of sampling occasions and positive results were very low. Due to the low prevalence of positive results no formal statistical methods were used to compare toxin concentrations between species. Summary statistics are presented instead for each species pair for which data exists, including the following parameters:

- The number of occasions on which dual testing of both species was undertaken;
- The number of sampling occasions on which toxins in both species were 'not detected';
- The number of sampling occasions on which toxins in both species were 'detected'; and
- The number of sampling occasions when toxins were detected in one species, but not the other.

The summary statistics were described for each of the regulated toxin groups.

No concurrent data was available for King scallops and mussels in the period 2010 – 2013. Given the implications in the literature regarding potentially higher levels of accumulation of ASP and PSP in scallops compared with mussels, the historical database between 2001 and 2010 was also scrutinised for historical ASP and PSP data for this species pair. Summary statistics as described above were prepared.

## 2.3 International use of indicator species: survey approach

To gain insight into the global use of indicator species in management programmes, a small survey was prepared and distributed to a number of shellfish producing countries. The questions focused on the species of shellfish used as indicators, the way in which indicators are used to inform management decisions, and the identification of issues relating to the use of indicator species. Appendix 2 contains a copy of the survey that was distributed as part of this project in 2014. Twelve countries responded to the survey including: Australia, Canada, England and Wales, France, Ireland, New Zealand, Northern Ireland, Portugal, Scotland, Sweden, the Netherlands and the USA. Responses to the survey are discussed in Section 4.

# SECTION THREE: LITERATURE REVIEW

Section 2.1 (Methodology) describes the approach taken to conduct the literature review and the search terms used.

## 3.1 Bivalve molluscan shellfish

Bivalve shellfish are a class of the phylum *Mollusca*. The class *Bivalvia* contains many different species of shellfish, including oysters, scallops, clams and mussels. The following section provides a summary of the filtration and feeding activities of bivalves, as this is relevant to considerations regarding potential interspecies differences in uptake and elimination of marine toxins and chemical contaminants. The following overview of bivalve feeding and filtration processes is primarily based on information contained within comprehensive reviews published by Yonge (1926), Morton (1983), Galtsoff (1964); Gosling (2003); Seamer (2007) and supplemented with additional information from more recent literature.

### 3.1.1 Bivalve feeding process

The basic anatomy of an oyster is depicted in Figure 3.1 as an example of the structure of a typical bivalve. Bivalve shellfish have two shells (valves) that are hinged together and the body is contained within the shell. The shell protects against predators and serves as a structure for the muscles to attach to. The mantle lies within the shells and encloses the bivalve body. The mantle plays a role in sensing the external environment, directing food particles for consumption or rejection, secretion of the shell and control of water flow. The gills lie within the mantle and their key function is as a respiratory organ, with gas exchange occurring across the thin gill walls between the haemolymph (blood) and the seawater. Re-oxygenated blood is then re-distributed throughout the bivalve body.

The other major role of the gills is in the capture, selection and transport of food particles. Cilia that are present on the gills create water currents, which move the water across the gills, and mucus on the gills binds particles present in the water. Particles bound in the mucus are then carried forward towards the labial palps. Mucus plays a major role in the processing of particles by bivalves. The functions of mucus are thought to be multiple, including: initial capture and transport of particles on the gills; transport of particles through the digestive tract; and transport of particles for rejection in pseudofaeces. Particles entrapped within mucus are directed along the palp to the mouth for ingestion, or along rejectory tracts to the inhalant opening where they are rejected as pseudofaeces (Ward et al., 1997, 1998). If food is supplied in excess, then the majority of particles are rejected.

Most bivalves can effectively retain particles that are 3-4 µm diameter, particles smaller than this may also be retained but at lower efficiencies. The size range of particles that can be effectively retained by scallops is around 5-7 µm; slightly higher than most bivalve species (reviewed in Gosling (2003); Shumway et al. (1985)). Particle selection and retention by bivalves is however a complex process: in addition to particle size, various other factors including charge and nutritional value have been postulated to influence food selection (Bedford et al., 1978; Shumway et al., 1985; Ward et al., 1997; Ward and Shumway, 2004). Accordingly, Gosling (2003) notes that "there is now increasing evidence to indicate that particle retention efficiency depends not just on particle size but also on shape, motility, density, and chemical cues such as algae ectocrines" and that bivalves can discriminate and select particles of high nutritional value and reject those of lower value.

This was demonstrated in a study in which 10 species of suspension feeding bivalves were fed a mixture of the algae *Phaeodactylum triconutum* and suspended material from the sea bottom. The results demonstrated that all 10 species were able to separate the algae from the silt and selectively ingest the algal component (Kiorboe and Mohlenberg, 1981). Kiorboe and Mohlenberg (1981) also noted that there was a positive correlation between the size of the labial palps and selection efficiency and that the "palps take part in the selection of particles". They further observed that different particles have different probabilities of being entrapped in the mucus, but that the mechanism of particle selection is unknown.

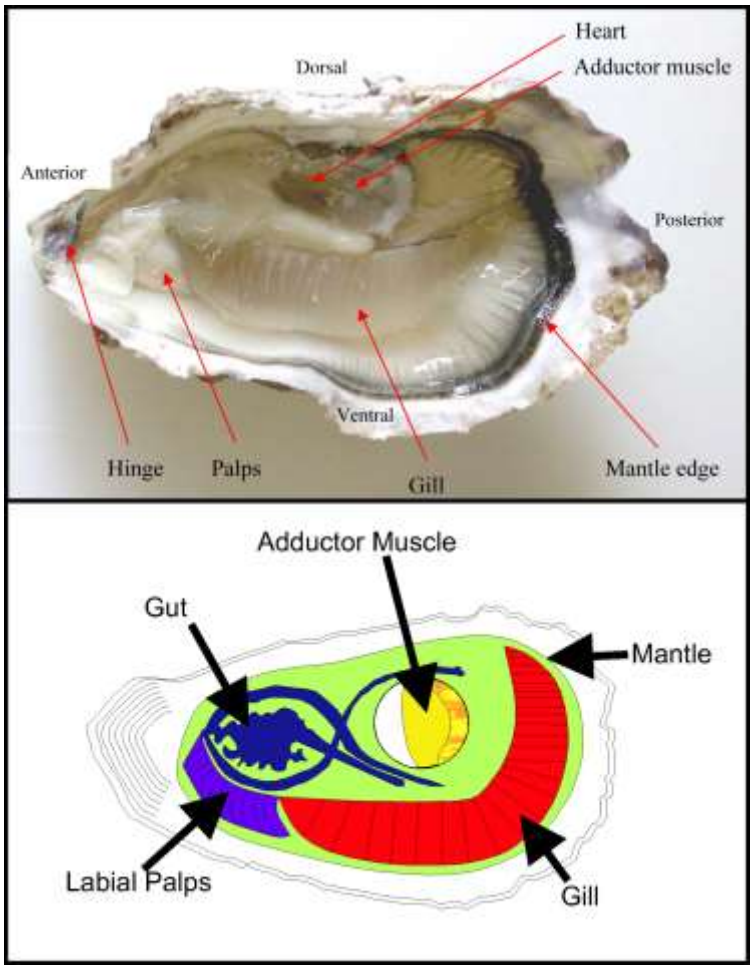


**Figure 3.1:** Anatomy of an oyster (photo credit A. Bell; Figure reproduced with permission from Seamer (2007))

Following the Kiorboe and Mohlenberg (1981) study, additional research demonstrated that bivalves can also distinguish between different types of similar sized algae, with some species preferentially selecting particular algal species for ingestion. Shumway et al. (1985) investigated the clearance of three different algal types (*Prorocentrum minimum, Phaeodactylum tricornutum* and *Chroomonas salina*) by six species of bivalves. The algae used in the study were of a similar size range. Key observations of the study included:

- The preferential clearance (retention) of *Prorocentrum minimum* cells by the oyster *Ostrea edulis;*
- The preferential rejection of the diatom *Phaeodactylum tricornutum* in the pseudofaeces of *Ensis directus*, *Placopecten magellanicus*, and *Artica islandica;* and
- The preferential absorption of *Chroomonas salina* by most of the bivalves.

The authors suggested that there are at least three mechanisms of particle selection in bivalves: (1) sorting on the gills; (2) pre-ingestive selection on the labial palps; and (3) preferential adsorption of some algal types following ingestion (Shumway et al., 1985).

Recently it has been demonstrated that components present on the cell surface of microalgae play a role in particle selection by the Eastern oyster (*Crassostrea virginica*), the King scallop (*Pecten maximus*) and the Pacific oyster (*Crassostrea gigas)*. Following this discovery, it was shown that carbohydrates present on the algal cell surface and lectins within mucus that cover the bivalve feeding organs specifically interact with each other (Espinosa et al., 2010a; Espinosa et al., 2009, 2010b). This was reported for both *Mytilus edulis* and *C. virginica*, and lectins within bivalve mucus were thus suggested to be a common mechanism for particle selection across bivalve taxa.

Particles that are selected for ingestion pass through the mouth into the oesophagus and then into the stomach. Food particles are then digested through both intra- and extra-cellular digestive processes. The crystalline style is located within the digestive tract and extends through the stomach with the end positioned within a sac at the end of the stomach. Cilia cause the style to revolve and mucus with food particles are wrapped around it as it turns. As the style revolves it rubs against the stomach, which causes the style to dissolve and secrete enzymes that facilitate the release of food from the mucus. The revolving action and released enzymes assist in the extracellular breakdown of food particles.

Food particles are also subjected to selective processes within the stomach, with large or unwanted particles passing from the stomach to the intestine and being excreted in the faeces (through the anus). The digestive diverticula (which consists of many tubules) branch off the stomach and are the main site of intracellular digestion. Waste products from intracellular digestion in the digestive diverticula are excreted into the lumen of the tubules, which are then passed to the intestine and voided along with other particles that have been rejected from the stomach.

### 3.1.2 Filtration and clearance rates

The volume of water that flows through the gills in a certain period of time is referred to as the 'filtration rate' (FR) and the volume of water that is cleared of particles in a particular period is referred to as the 'clearance rate' (CR) (Gosling, 2003; Riisgard, 2001). The propensity of bivalves to accumulate marine toxins and chemical contaminants may be largely reliant on filtration and clearance rates, and a bivalve species ability to ingest or absorb a particular algae and toxin type. The following section therefore provides an overview of the major factors that influence the FR and CR of bivalves.

#### *Interspecies variation*

There is variation in FR and CR both between and within species. However, some of the variation noted has been suggested to be related to methodological differences in experimental design used for studies, including the use of different methods to measure rates, incorrect use of measurement methods, differences in shell length/body weight ratio and disparities in the quality and quantity of the food available (Gosling, 2003; Riisgard, 2001). Generally, the FR and CR of bivalves has been found to be associated with the body size, with rates declining for animals of larger size (Gosling, 2003). Despite the variation related to these variables, there still appears to be inter- and intraspecies variation in rates – as observed through studies which investigate FR and CR in multiple species using the same experimental design.

Kiorboe and Mohlenberg (1981) presented CR data for nine different species of suspension feeding bivalves using the same method, some data was generated by the authors themselves and others by Møhlenberg and Riisgård (1979). The data presented by Kiorboe and Mohlenberg (1981) suggested that the CR of cockles (*C. edule*) and queen scallops (*A. opercularis*) were higher than the CR of Pacific oysters (*C. gigas*) and the common mussel (*M. edulis*) (Table 3.1). Riisgard (2001) further summarised the filtration rates of suspension feeding bivalves and tabulated so-called 'reliable' rates recorded for bivalves using the various methodologies (reproduced in Gosling (2003)). The summary produced by Riisgard (2001) also suggests that for bivalves of the same body weight, that cockles (*C. edule*) have a higher FR than other species of bivalves examined in the same experiments, including *M. edulis.* The Riisgaard data also suggested that the scallop *Chlamys hastata* had a high FR, which exceeded that of *M. edulis* and the Californian mussel (*Mytilus californianus*) (Riisgard, 2001).

**Table 3.1:** Clearance rates of nine species of bivalves for individual shellfish of 1 cm shell length. Mussel values represent collections from two different sites: The Sound[a] and The Wadden Sea[b]. Data reproduced from Kiorboe and Mohlenberg (1981) and Møhlenberg and Riisgård (1979).

| Species | Common name | Species commercially produced in Scotland | Clearance (ml/min) |
|---|---|---|---|
| *Cerastoderma edule* | Cockles | ✔ | 6.89 |
| *Aequipecten opercularis* | Queen scallops | ✔ | 5.61 |
| *Acanthocardia echinata* | Prickly cockle | | 4.90 |
| *Crassostrea gigas* | Pacific oysters | ✔ | 3.84 |
| *Artica islandica* | Icelandic cyprine | | 3.79 |
| *Mytilus edulis* | Blue mussel or common mussel | ✔ | 3.36[a]<br>2.75[b] |
| *Spisula subtruncata* | Cut trough shell | | 2.90 |
| *Mya arenaria* | Soft shelled clam | | 1.85 |
| *Musculus niger* | Black mussel | | 0.49 |

Several more recent studies that compare FR and/or CR between bivalve species within the same study (thus utilising the same experimental design) are briefly summarised below:

- Gardner (2002) compared the CR of three mussel genera (*Mytilus galloprovincialis*, *Perna canaliculus* and *Aulacomya maoriana*) that co-occur in New Zealand, over a range of ambient seston conditions. Significant differences in CR were observed between the three species, with *M. galloprovincialis* having the highest CR of the three species.
- Filtration rates of different size groups of *C. edule*, *M. edulis* and *M. arenaria* (soft-shelled clams) in the presence of algal cells was investigated and individual mean FR values ranged between 0.4 – 3.7, 1.2 – 4.3, 1.2 – 3.8 L/hour for each species respectively (Riisgard et al., 2003).
- Clearance rates of *P. maximus* and *M. edulis* were investigated in a laboratory study involving exposure to four different treatments composed of various concentrations of natural seston. The mean CR for *P. maximus* for all treatments was between 22 and 32 L/h. The mean CR for *M. edulis* was between 2.6 and 4.5 L/h (Strohmeier et al., 2009).

Collectively the results of these studies suggest that when treatment (or experimental) conditions are identical that cockles and scallops may have FR and CR that exceed those of

the mussel *M. edulis*. The noted between species variation in FR and CR could potentially lead to higher levels of toxin accumulation in some bivalve species and may account for the observed differences in marine toxin concentrations of different bivalve species grown in the same areas.

Supporting this concept, a study undertaken by Li et al. (2001) involved feeding a mixture of the non-toxic diatom, *Thalasiosira pseudonana,* and the PSP-producing dinoflagellate, *Alexandrium tamarense,* to scallops (*Chlamys nobilis*) and clams (*Ruditapes philippinarum*). The maximum CR for scallops was found to be two times higher than that of the clams, and scallops were also found to have a higher adsorption efficiency (AE) of *A. tamarense* than clams. The authors noted that scallops in the South China Sea contain higher levels of PST than clams, and suggest that the difference in toxin burden may be related to the observed differences in feeding and adsorption behaviour (Li et al., 2001). Differences in toxin accumulation between bivalve species are discussed further in Section 3.3.4.

While there is marked interspecies variation when environmental conditions are the same, several additional factors also impact the FR and CR of bivalves, including the quality and quantity of food available and seawater temperature and salinity. The alteration of bivalve response to these additional factors emphasises the complexity of bivalve FR and CR and difficulties in making generalisations and interspecies comparisons.

### *Impact of food concentration*

In general terms, studies have demonstrated that the FR and CR increases with food availability to a certain level, then declines with further increases in food concentration (Barille et al., 1997; Pascoe et al., 2009; Riisgard et al., 2003; Strohmeier et al., 2009). A study undertaken by Barille et al. (1997) on the Pacific oyster *(C. gigas*) showed that FR increased with increasing concentrations of suspended particulate matter up to 90 mg/L. Seston concentrations greater than 90 mg/L resulted in a reduction in FR. Similarly, *P. maximus* and *M. edulis* were shown to have high CR at low seston quantities, which increased with food availability and then declined with further increases in food quantity (Strohmeier et al., 2009).

It has also been determined that when seston concentration drops below a critical level, that filtration activity ceases altogether; experiments undertaken on actively filtering *M. edulis* and *C. edule* demonstrated that decreasing algal quantities below a certain level led to cessation of filtering. The addition of more food restored filtration activity (Riisgard et al., 2003). Similar results were obtained by Pascoe et al. (2009) in experiments in which *M. edulis* was fed on cultured *Isochrysis galbana*. Results demonstrated a trigger level for algal cell concentration, below which filtering ceased, above this level CR increased to a maximum level and then decreased if additional algal cells were added (Pascoe et al., 2009). In addition to seston concentration, it has been reported that the CR of various mussel species and the scallop *Chlamys farreri* is also modulated in response to the quality and composition of available food (Gardner, 2002; Hawkins et al., 2001).

### *Impact of seawater temperature and salinity*

Generally, increasing water temperatures are reported to result in elevated FR and CR (Gosling, 2003). This has been reported for several species of shellfish, including the Queen scallop, *A. opercularis,* and the mussel, *M. edulis* (reviewed in Gosling). Haure et al. (1998) demonstrated that the CR of the native oyster, *O. edulis*, increased as the temperature increased (up to 30°C) when fed *Skeletonema costatum*. The Pacific oyster, *C. gigas*, also showed an increase in CR when temperatures increased between 5 and 20°C, however, CR

decreased when temperatures were further increased from 20°C to 32°C when fed a diet of *I. galbana* and *Chaetoceros calcitrans.* Anestis et al. (2010) demonstrated that the CR of *M. galloprovincialis* decreased significantly when they were maintained at 24°C, 26°C or 28°C when compared to mussels maintained at 18°C.

Oysters and mussels are euryhaline (can tolerate a wide range of salinities), thus are able to cope with very low salinities, through to full marine conditions. It is suggested, however, that when they are transferred to salinities that they are not acclimated to, that the FR and CR reduces significantly (reviewed in Gosling (2003)). The effects of various seawater salinities (between 12 and 34 psu) on the feeding activity of oyster spat (*O. edulis*) from Sweden were examined (Rodstrom and Jonsson, 2000). The results showed that the feeding rate began to decline at salinities below 28 psu and ceased at 16 psu. At salinities less than 16 psu spat did not regain feeding activity when returned to fully saline waters. The CR was noted to decline at salinities less than 24 psu (Rodstrom and Jonsson, 2000). Scallops appear intolerant of low salinities – seemingly preferring oceanic conditions as reflected by their subtidal habitat. For example, the observed CR for King scallop spat (*P. maximus*) held in the salinity range of 30 – 35 (fully marine) were found to be similar, however, lower CRs were consistently observed for scallop spat held at 26 psu when compared to 30 psu. Additionally, mortalities were observed for spat held at 20 psu (and 10°C) (Laing, 2002).

### 3.1.3 Bivalve shellfish species commercially harvested in Scotland

The bivalve shellfish species that are commercially produced in Scotland are shown in Table 3.2 along with associated volume (tonnes) harvested. The value of commercially harvested shellfish species is presented in Table 3.3. Mussels (*Mytilus* spp.) are the most significant commercially produced bivalve species in Scotland by volume and value, followed by razors (*Ensis* sp.) and Pacific oysters (*C. gigas*). Small volumes of cockles (*C. edule),* surf clams (*Spisula solida*), native oysters (*O. edulis*) and cultivated King and Queen scallops (*P. maximus* and *A. opercularis* respectively) are also commercially harvested from the in-shore environment. Scallops that are harvested from off-shore sites are not included in the 'volume and value' summary tables (Tables 3.2 and 3.3), as they have alternate arrangements in place for toxin and contaminant management (testing through processors or dispatchers), and are thus outside the scope of the review. It is also noted that there is one classified production area for wedge clams *(Donax vittatus)* in Scotland*,* however production volumes do not appear in currently published fisheries statistics and they are also therefore omitted from Tables 3.2 and 3.3.

The marine habitat that commercial bivalve species occupy is important to any discussion on appropriate indicator bivalve species, as species which occupy distinctly different habitats may be exposed to different populations of algae and harmful algal blooms, which could in turn result in significant interspecies differences in toxin accumulation. Therefore, the following section briefly describes the habitat that the major commercial bivalve species occupy. Table 3.4 presents a comparison of the habitat, harvesting and production methods, and temperature and salinity ranges for the major commercial bivalve species in Scotland. Other authors have reviewed the habitat of commercial bivalve species (Breen et al., 2011; Gosling, 2003; Laing and Spencer, 2006; SeaFish, 2002), additionally the Biological Traits Information Catalogue (BIOTIC) compiled by the Marine Life Information Network provides comprehensive habitat information for bivalve species (MarLIN, 2006), therefore these have been used as the primary source of information for the proceeding sections and Table 3.4.

**Table 3.2:** Volume (tonnes) of commercially produced bivalve shellfish in Scotland: 2008 – 2012[a]

| | **Mussels** | **Razors** | **Pacific oysters** | **Cockles** | **Surf clams** | **Native oysters** | **Queen scallop[b]** | **King Scallop[b]** |
|---|---|---|---|---|---|---|---|---|
| | *Mytilus* spp. | *Ensis* sp. | *Crassostrea gigas* | *Cerastoderma edule* | *Spisula solida* | *Ostrea edulis* | *Aequipecten opercularis* | *Pecten maximus* |
| 2008 | 5869 | 526 | 248 | 6 | 101 | 20 | 27 | 2 |
| 2009 | 6302 | 718 | 232 | 9 | 94 | 39 | 6 | 4 |
| 2010 | 7199 | 666 | 241 | 342 | 108 | 28 | 7 | 8 |
| 2011 | 6996 | 719 | 251 | 0 | 40 | 28 | 1 | 9 |
| 2012 | 6277 | 900 | 216 | 5 | 6 | 25 | 0.4 | 7 |
| **5 Year Average** | **6529** | **706** | **238** | **72** | **70** | **28** | **8** | **6** |

[a]Data sources: (Anonymous, 2013; Mayes and Fraser, 2010, 2011, 2012; McCurchie and Fraser, 2009; Munro et al., 2013)
[b]Volume of scallops harvested is for cultivated scallops only. This does not include those directly harvested for human consumption from off-shore fisheries.

**Table 3.3:** Value (£ 000,000) of commercially produced bivalve shellfish in Scotland: 2008 – 2012[a]

| | **Mussels** | **Razors** | **Pacific oysters** | **Cockles** | **Surf clams** | **Native oysters** | **Queen scallop[b]** | **King Scallop[b]** |
|---|---|---|---|---|---|---|---|---|
| | *Mytilus* spp. | *Ensis* sp. | *Crassostrea gigas* | *Cerastoderma edule* | *Spisula solida* | *Ostrea edulis* | *Aequipecten opercularis* | *Pecten maximus* |
| 2008 | 5.9 | 1.5 | 1.5 | 0.006 | 0.14 | 0.09 | 0.05 | 0.01 |
| 2009 | 6.3 | 1.8 | 1.2 | 0.02 | 0.11 | 0.13 | 0.01 | 0.02 |
| 2010 | 6.7 | 1.8 | 1 | 0.32 | 0.15 | 0.1 | 0.03 | 0.05 |
| 2011 | 8.3 | 2 | 1.25 | 0 | 0.06 | 0.14 | 0.003 | 0.09 |
| 2012 | 7.5 | 2.6 | 0.95 | 0.01 | 0.01 | 0.19 | 0.001 | 0.1 |
| **5 Year Average** | **6.94** | **1.94** | **1.18** | **0.0712** | **0.094** | **0.13** | **0.0188** | **0.054** |

[a]Data sources: (Anonymous, 2013; Mayes and Fraser, 2010, 2011, 2012; McCurchie and Fraser, 2009; Munro et al., 2013)
[b]Volume of scallops harvested is for cultivated scallops only. This does not include those directly harvested for human consumption from off-shore fisheries.

### *Mussels*

Mussels grow in the intertidal to subtidal regions of rocky shores. They can grow in estuarine to fully marine seawater, in sheltered and exposed sites and they attach to a wide range of substrates, including rocks, wood, ropes, and shells (reviewed in Gosling (2003)). In Scotland, mussels are primarily commercially grown using suspended cultivation methods, in some cases they are also cultivated on the seabed. Suspended cultivation is mostly rope culture on longlines. *M. edulis* is reported to be tolerant of salinities that range between 4 and 40 psu (reviewed in Gosling (2003)). *M. edulis* is also reported to be tolerant of wide temperature ranges: Water temperatures between 10 – 20°C had little impact on growth of *M. edulis* and they are also reported to be reasonably tolerant of cold and freezing (reviewed in MarLIN (2006)).

Commercial mussel production in Scotland has been traditionally based on the common mussel, *M. edulis. M. galloprovincialis*, the Mediterranean mussel, has however expanded out of the Mediterranean area and been observed in Scotland, including the Shetlands in the far north. A third closely related mussel species, *Mytilus trossulus*, was considered to be restricted to the Baltic sea. Mussels with fragile shells had been reported in Loch Etive in Scotland, this prompted an investigation in 2006 which revealed the presence of all three mussel species in Loch Etive (*M. edulis, M. galloprovincialis* and *M. trossulus*) and their hybrids (Beaumont et al., 2008). A subsequent study in Loch Etive in 2007 showed that the frequency of *M. galloprovincialis* and its hybrids was low, however *M. trossulus* (37%) was more common than *M. edulis* (30%) and their hybrids (23%) (Dias et al., 2009a). A survey of 41 mussel farms in Scotland was subsequently undertaken in late 2007 which utilised quantitative PCR to differentiate the three species. This revealed *M. edulis* alleles were present in samples collected from all 41 sites, *M. galloprovincialis* alleles at 39 sites, and *M. trossulus* alleles at five sites (Dias et al., 2009b).

### *Oysters*

Oysters readily grow in the intertidal and subtidal zones, however they generally prefer growing in estuarine areas. Pacific oysters are cultivated in Scotland in trays or bags on trestles on the foreshore, native oysters are cultivated on substrate on the seabed. The optimum salinity for Pacific oysters is in the range of 23-28 psu (reviewed in (Gosling, 2003)). Oyster species are extremely tolerant of wide salinity ranges (e.g. euryhaline), for example, *C. virginica* is reported to grow in salinities ranging between 4 and 40 psu, but has a salinity optimum of 14 – 28 psu (Gosling, 2003). Native oysters may be less tolerant of lower salinities than other species e.g. Rodstrom and Jonsson (2000) noted that feeding rates of native oyster spat (*O. edulis*) decreased at 28 psu and ceased at 16 psu.

### *Clams*

Gosling (2003) notes "*Of the four bivalve groups, clams occupy the broadest range of habitats…..they are found from open coast to sheltered, saline and estuarine locations*". Clams can inhabit the intertidal and subtidal zones, and grow at a range of depths within a variety of substrates, including sand, mud and gravel. Clams are commercially harvested using a variety of techniques that involve diving, dredging or hand harvesting. There are two species of razors of commercial importance in Scotland: *Ensis siliqua* and *Ensis arcuatus,* which are reported to be located from the lower shore to around 20 m depth (Breen et al., 2011; SeaFish, 2002). Surf clams (*S. solida)* are harvested in the open ocean to water depths of 30 m and are covered in around 5-15 cm of substrate (Gosling, 2003). Cockles (*C. edule)* are harvested from the mid tide to the low water mark and are covered with around 5 cm of substrate (Gosling, 2003). They are reported to be intolerant of low temperatures (with reduced clearance rates suggested) and winter mortalities have been noted (MarLIN, 2006).

*C. edule* and *S. solida* are euryhaline species, tolerating salinities in the range of 18 to 40 psu.

### *Scallops*

In Scotland King (*P. maximus*) and Queen scallops (*A. opercularis*) are commercially harvested from off-shore sites via diving and trawling. King and Queen scallops are generally located in fully marine waters from below the low water mark to 180 m and between 20 and 100 m respectively. These scallops are directly sold for human consumption and are subject to different controls with respect to marine biotoxin and contaminants (testing through processors or dispatchers) than other bivalve species that are cultured or harvested from wild stocks in the in-shore environment. In some cases, spat are harvested from the off-shore environment and then brought to in-shore areas for on growing in suspended bags (Queen and King scallops) or on Several Orders (legislation which grants exclusive fishing rights within a designated area) on the seabed (King scallops). Currently there are two in-shore areas that are classified for scallops in Scotland. As noted previously, scallops are not particularly tolerant of low salinity water, research undertaken on King scallop spat demonstrated a lower CR for scallops held at 26 psu compared with scallops held in fully marine conditions (30 – 35 psu) (Laing, 2002).

**Table 3.4:** Preferred habitat and production/harvesting methods for commercially produced bivalves in Scotland[a]

| | **Mussels** | **Razors** | **Pacific oysters** | **Cockles** | **Surf clams** | **Native oysters** | **Queen scallop[b]** | **King Scallop[b]** |
|---|---|---|---|---|---|---|---|---|
| | *Mytilus* spp. | *Ensis* sp. | *Crassostrea gigas* | *Cerastoderma edule* | *Spisula solida* | *Ostrea edulis* | *Aequipecten opercularis* | *Pecten maximus* |
| Preferred habitat | Intertidal to subtidal zones of rocky shores and estuaries | Lower shore to shallow sublittoral zone. *E. siliqua*: 0- 20 m (fine sands and mud) *E. arcuatus*: 0-42 m (course grained sediments) | Intertidal to subtidal zones of estuaries | From mid-tide to low water line. Grow in sand, mud or gravel substrates. Burial depth <5cm. | Open ocean to 30m. Grows within sand substrate. Burial depths of 5-15cm. | Shallow sheltered estuarine waters | Grows at depths of 20-100m (natural off-shore habitat) | Below low water mark to 180m. Prefers clean firm sand or fine gravel/mud. (natural off-shore habitat) |
| Salinity | Euryhaline: *M. edulis* tolerates 4-40 psu | Fully marine[c] | Euryhaline: suggested optima of 23-28 psu | Euryhaline: 18 – 40 psu | Euryhaline: 12 – 35 psu | Euryhaline | Fully marine[c] | Fully marine[c] |
| Aquaculture or fishery product | Aquaculture | Fishery | Aquaculture | Fishery | Fishery | Fishery | Aquaculture | Aquaculture |
| Production and/or harvesting method in Scotland | Seabed and suspended cultivation. Mostly rope culture on longlines | Hand digging Diving Dredging | Cultivated in trays or bags on trestles on the foreshore | Hydraulic suction dredge (not on foreshore) Hand raking | Dredging Hand harvesting | Bottom culture, directly onto substrate | Suspended cultivation | Suspended and seabed cultivation |

[a]Information in this table has been synthesised from Gosling (2003); Laing and Spencer (2006); MarLIN (2006), Breen et al. (2011); SeaFish (2002).
[b]Suspended and seabed cultivation takes place in the in-shore environment. Scallop spat is harvested off-shore and then brought to the in-shore areas for on growing in suspended bags (Queen and King scallops) or on Several Orders on the seabed (King scallops). Scallops are also harvested directly for human consumption from the off-shore environment – but are not considered in this summary (as this is outside the scope of the review).
[c]Gosling (2003) notes that fully marine conditions are considered to be between 32 – 38 psu.

## 3.2 Use of indicator (or sentinel) species to manage biotoxin and chemical contaminant risks related to shellfish

A sentinel is by definition a person who keeps watch and provides warning of ensuing dangers. The use of animal sentinel species (synonymous with the term indicator species) in the oceans has been adopted widely as a common approach to warn of incumbent risks to oceans and humans that rely on the sea for food and recreation. An indicator species is one that may give early warning of an emerging health hazard from the ocean environment. Indicator species are generally considered "sensitive….of a chemical contaminant, biological toxin or pathogen due to their ability to concentrate or integrate exposures within a food web ecosystem" (Schwacke et al., 2013).

Use of appropriate bivalve shellfish indicator species in the marine environment may provide forewarning of the presence of toxins and chemical contaminants in a range of shellfish species that are growing in the same habitat, and thus provide an opportunity to limit the amount of shellfish that is marketed and consumed and any ensuing human health risk.

Bivalve shellfish indicator species are frequently used within risk management programmes that aim to limit and reduce the risks to consumers of bivalve shellfish from marine toxins. Indicator species are routinely monitored for a range of marine toxins in areas in which multiple bivalve species are cultivated or wild-harvested. This approach is often taken to reduce the costs associated with testing all species from each production area. If the indicator species within a production area is found to be positive at a critical (threshold) level, this can trigger a variety of management responses, including cessation of harvesting of all species in the area. The use of indicator species by a range of shellfish producing countries, and management responses that may be taken, are discussed further in Section 4.

Indicator species need to be carefully selected to ensure that they are sensitive to potential toxins and contaminants. Schwacke et al. (2013) proposed a series of criteria[5] to assist in judging the appropriateness of a given species as an indicator, including:

1. **Sensitivity for bioaccumulation**: *Indicator species should be highly sensitive to concentration of the toxin or contaminant, and be more sensitive than the other shellfish species of interest;*
2. **Appropriate distribution**: *Indicator species should have a distribution that overlaps with the other shellfish species of interest;*
3. **Indicator on an appropriate scale**: *The indicator should be chosen based on its ability to provide representative information on the appropriate scale (or to the specified region in which it is used);*
4. **Ease of sampling**: *Samples can be easily collected;*
5. **Existing infrastructure and protocols for consistent collection, analysis and data archiving**: *A consistent sampling approach will ensure spatial and temporal consistency and allow for comparisons to be made, and long term trends to be analysed.*

[5] The definitions of the criteria have been modified to suit shellfish/biotoxin/chemical contaminant applications.

Schwacke et al. (2013) noted that meeting as many of these criteria as possible provides the optimal opportunity for assessing human health impacts adequately, but that "meeting all criteria is generally not possible".

In July 2002, the 25th session of the Codex Committee on Fish and Fishery Products (CCFFP) requested the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to provide scientific advice on biotoxins to assist in the development of standards for bivalve molluscs. In 2004, three expert working groups were established under the auspices of the FAO and WHO to provide the advice requested by the CCFFP, this advice was formally published by the FAO in 2011. The advice provided includes the following statement regarding the use of indicator shellfish species:

***"The selection of an indicator shellfish species for each toxin group is problematic because the rate of toxin uptake and depuration is unique to the combination of species, toxin and geographic location. It is important to note that, using an indicator shellfish species, the absence of toxicity in the indicator species is assumed to imply the absence of toxicity in other species in the growing area. This implication must be verified for each shellfish species and for each group of toxins before defining a particular shellfish species as an indicator for that growing area. (Lawrence et al., 2011)"***

This advice should be considered in conjunction with the criteria established by Schwacke et al. (2013) regarding selection of appropriate indicator species to use in risk management programmes.

## 3.3 Marine toxins

### 3.3.1 The toxins and toxin producers

Marine toxins are a diverse group of chemicals that are produced by a variety of harmful algae, bivalve shellfish feed on algae, including the toxin-producing dinoflagellates, and diatoms. This can lead to accumulation of a large variety of toxins within the digestive and other tissues of bivalves. If significant quantities of toxins are accumulated, people who consume the shellfish may become ill. In order to protect human health, shellfish are routinely monitored for a variety of toxins and harvesting restrictions are applied if levels are found to exceed critical limits. In Scotland the regulated marine biotoxin groups are[6]:

- Paralytic shellfish poisons (PSP) (synonymous with saxitoxin-group toxins and paralytic shellfish toxins)
- Amnesic shellfish poisons (ASP) (synonymous with domoic acid-group toxins)
- Diarrhetic shellfish poisons (DSP) (comprises okadaic acid, dinophysistoxins and pectenotoxins together)
- Yessotoxins
- Azaspiracids

The following sections provide a brief overview of the defining characteristics of each the regulated groups noted above. In recent years several reviews of marine toxins in bivalves have been completed by the WHO/FAO and by the European Food Safety Authority (EFSA). These provide comprehensive details on the toxins that comprise each group, their effects in

[6]In 2004 an FAO/WHO expert working group proposed that nomenclature of toxin groups be based on chemical structure. In this report however, the nomenclature used is based on the current toxin groups that are regulated in Scotland and the commonly used terminology.

humans and animals, mode of action, cases/outbreaks and toxicity etc (EFSA, 2008a, 2008b, 2009a, 2009b, 2009d, 2010b; Lawrence et al., 2011; Paredes et al., 2011; Toyofuku, 2006). These recent reviews have been used as the basis of information presented below.

### *Paralytic shellfish poisons (PSP)*

- **The toxins:** PSPs are a group of non-proteinaceous toxins composed of related congeners that have been identified in toxic algae (predominantly dinoflagellates) and various species of seafood (EFSA, 2009d; van Egmond et al., 2004; Wiese et al., 2010). Saxitoxin, the first PSP toxin discovered, consists of a 3,4-propinioperhydropurine tricyclic structure and it has the molecular formula $C_{10}H_{17}N_7O_4$. Since the discovery of saxitoxin in 1957, a further 57 different analogues have been identified from various organisms (Wiese et al., 2010).

- **The toxin producers:** PSPs are produced by some species of marine dinoflagellates in the genera *Alexandrium*, *Gymnodinium* and *Pyrodinium*. Four species of *Alexandrium* have been detected in Scottish waters: *A. tamarense*, *A. minutum*, *A. ostenfeldii* and *A. tamutum* (Swan and Davidson, 2012). *A. tamarense* is the most commonly reported and both toxic and nontoxic variants are found in Scottish waters (Collins et al. 2009; Touzet et al., 2010). Cultures of *A. minutum* and *A. tamatum* grown from cells isolated from Scottish waters did not produce PSP (Brown et al 2010). *Gymnodinium catenatum* and *Pyrodinium bahamense var*. *compressum* have not been detected in Scotland (Swan and Davidson, 2012). PSP production has also been linked to some freshwater cyanobacteria in the genera *Anabaena*, *Aphanizomenon, Cylindrospermopsis, Lyngbya* and *Planktothrix* (Mulvenna et al., 2012; Pearson et al., 2010; Wiese et al., 2010).

- **Symptoms:** Nausea, paraesthesia, tachycardia, muscular paralysis, respiratory failure and death (Munday and Reeve, 2013).

- **Cases/outbreaks:** Reports of PSP illnesses date back to the 18$^{th}$ century and since the 1940s cases have been recorded worldwide, including in north and south America, parts of Asia, Australia and New Zealand, Africa and Europe (EFSA, 2009d). Outbreaks of shellfish poisoning of humans have also occurred in the UK, with around 10 outbreaks noted up to 1975 (Hinder et al., 2011; Joint et al., 1997). In 2009, EFSA reviewed and summarised records of illnesses in which quantitative information is available (several hundred cases) (EFSA, 2009d).

- **Mode of action:** PSPs bind to the voltage gated sodium channels which blocks the inward flow of $Na^+$ to the cell. This inhibits action potential and prevents nerve transmission impulses (or 'signals' being passed from cell to cell). This is what leads to the reported paralytic effects of PSP in humans e.g. muscular paralysis, respiratory distress etc. (Anon, 2001; Cestele and Catterall, 2000; Munday and Reeve, 2013; Zhang et al., 2013)

- **Toxicity to animals:** The carbamate and decarbamoyl saxitoxin analogues are reported to be the most toxic, with potency of the various analogues varying significantly. Saxitoxin is reported to be around 100 times less potent when administered via the oral route (Toyofuku, 2006).

- **Maximum permissible level:** Regulation (EC) No 853/2004 states that bivalve molluscs placed on the market for human consumption must not contain PSP at levels exceeding 800 µg/kg.

### *Diarrhetic shellfish poisoning (DSP): Okadaic acid (OA), dinophysistoxins (DTXs) and pectenotoxins (PTXs)*

- **The toxins:** These are heat-stable polyether compounds that are lipophilic. The compounds include the parent molecule okadaic acid (OA) and its isomer 19-epi-okadaic acid, the OA analogues dinophysistoxin-1 (DTX1) and dinophysistoxin 2 (DTX2), and the acylated derivatives of OA, DTX1 and DTX2 – which are collectively known as DTX3 (EFSA, 2008a). Several diol-esters named dinophysistoxins 4 and 5 have also been isolated from *Prorocentrum* species (Lawrence et al., 2011). Pectenotoxins are polyether lactone compounds that often co-occur with OA and DTXs. Approximately 15 PTX analogues had been isolated and characterised up until 2009 (EFSA, 2009a).

- **The toxin producers:** OA and DTXs are produced by several dinoflagellate species of the genus Dinophysis (*Dinophysis acuta, D. acuminata, D. fortii*) and Prorocentrum (e.g. *Prorocentrum lima, P. hoffmanianum, P. concavum, P. belizeanum, P. rhathymum*). PTXs are considered to be produced exclusively by dinoflagellates of the genus *Dinophysis* (reviewed in EFSA (2009a); Paredes et al. (2011)). The IOC-UNESCO list 11 species of *Dinophysis* that are potential DSP producers and six species have been identified in Scotland. *D. acuminata* is reported to be the most commonly observed species in Scotland (Swan and Davidson, 2012). *P. lima* has also been identified in Scotland. *Dinophysis* species are planktonic, but *Prorocentrum* species are benthic or epibenthic species that are periodically detected in the water column.

- **Symptoms:** The predominant symptoms associated with analogues of OA and DTX are: diarrhoea, nausea, vomiting and abdominal pain. Fever, chills and headaches have also been reported in some cases (EFSA, 2008a). There have been no reports of human illness related to exposure to PTXs (EFSA, 2008a; Toyofuku, 2006).

- **Cases/outbreaks:** Illnesses associated with OA and its analogues in shellfish have been reported since the 1970s and have occurred worldwide. Several outbreaks of illness have also originated from consumption of Scottish shellfish, including a recent illness outbreak in 2013[7]. There have been no reports of human illness or symptoms related to exposure to PTXs (EFSA, 2008a; Munday and Reeve, 2013; Toyofuku, 2006).

- **Mode of action:** The mode of action of OA and its analogues is uncertain. Protein phosphatases have been shown to be inhibited *in vitro* and have been implicated in causing diarrhoea, however Munday and Reeve (2013) note that there is no *in vivo* evidence of this. Mode of action of PTXs is unknown (Munday and Reeve, 2013).

- **Toxicity to animals:** The lethal dose obtained by intra peritoneal administration of OA and DTXs is three to six times lower than by oral administration. Okadaic acid has been shown to be genotoxic to various cell types *in vitro* (Toyofuku, 2006). Okadaic acid and DTX1 are considered tumour promoters in animals (Munday and Reeve, 2013). Pectenotoxins have been shown to be acutely toxic to animals by the intra-peritoneal route. Several studies have also been undertaken on oral toxicity, which show conflicting results: one study suggested similar toxicity to the intra-peritoneal route, and other studies show negligible toxicity (Lawrence et al., 2011). There is no evidence of toxic effects in humans (Munday and Reeve, 2013).

- **Maximum permissible level:** Regulation (EC) No 853/2004 states that bivalve molluscs placed on the market for human consumption must not contain OA, DTXs and PTXs together at levels exceeding 160 µg/kg of OA equivalents.

[7] http://www.food.gov.uk/news-updates/news/2013/jul/shellfish#.U51th41dUSs

### *Azaspiracids (AZA)*

- **The toxins:** Azaspiracids were initially detected in mussels from Killary Harbour in Ireland in 1995. They are nitrogen-containing polyether toxins which contain a cyclic imine, a spiral ring and a carboxylic acid (Lawrence et al., 2011). In 2008, LC-MS/MS analysis had identified up to 27 different naturally occurring congeners of AZA1 (Twiner et al., 2008).
- **The toxin producer:** In 2008, the purported primary producer of AZA was isolated from seawater samples from the North East coast of Scotland. The organism was a small (7 – 11 µm) marine dinoflagellate called *Azadinium spinosum* (Salas et al., 2011; Tillmann et al., 2009). *A. spinosum* from Scotland and from Ireland have been reported to primarily produce AZA1 and AZA2 (Salas et al., 2011). Recently a linkage between *A. spinosum* and accumulation of AZA in mussels has been demonstrated through feeding studies (Jauffrais et al., 2012b; Salas et al., 2011), this was an important progression as toxicity in mussels has not always clearly correlated with the presence of *A. spinosum* in water samples (perhaps due to its small size and reported difficulties in identification using light microscopy based methods). It has been recently noted however, that additional food web components may also be involved in AZA accumulation (e.g. heterotrophic dinoflagellates), but this remains to be tested (Tillmann et al., 2014).
- **Symptoms:** Nausea, vomiting, diarrhoea, stomach cramps. Illness appears within hours of ingestion and persists for two to three days.
- **Cases/outbreaks:** Up until 2008, five illness outbreaks had occurred, all of which were related to shellfish produced in Ireland. Four of the outbreaks were related to mussel consumption and one to King scallops (Twiner et al., 2008; James et al., 2004). An additional illness outbreak was reported to occur in 2013 relating to mussels produced in Ireland that were processed in the Netherlands[8].
- **Mode of action**: Unknown (Munday and Reeve, 2013; Paredes et al., 2011).
- **Toxicity to animals**: AZA2 and 3 are reported to be more toxic than AZA1 by the intraperitoneal route. No oral toxicity data is available. A long-term experiment indicates that AZA may be carcinogenic or a tumour promoter, however this has not been proven using standard testing methodology (Munday and Reeve, 2013; Toyofuku, 2006).
- **Maximum permissible level:** Regulation (EC) No 853/2004 states that bivalve molluscs placed on the market for human consumption must not contain AZAs at levels exceeding 160 µg/kg of azaspiracid equivalents. Regulation (EC) No 2074/2005 requires that methods must at least be able to detect the analogues AZA1, AZA2, and AZA3.

### *Amnesic shellfish poisons (ASP)*

- **The toxins:** The major compound in the ASP toxin group is domoic acid (DA), which is a water-soluble cyclic amino acid. Several isomers of DA have also been reported (e.g. epi-domoic acid and iso-domoic acids A-H), but not all have been detected in shellfish (EFSA, 2009b).
- **The toxin producers:** Diatoms in the genus *Pseudo-nitzschia* produce DA e.g. *Pseudo-nitzschia multiseries* (formerly *Nitzschia pungens f. multiseries*), *P. australis*, *P. seriata*, and *P. pungens*. Eleven species of *Pseudo-nitzschia* have been reported to produce DA globally, nine species of *Pseudo-nitzschia* have been identified in Scotland. Testing to

[8] https://webgate.ec.europa.eu/rasff-window/portal/index.cfm?event=notificationDetail&NOTIF_REFERENCE=2013.1596

date confirms that *P. australis* and *P. seriata* in Scotland are DA producers (Swan and Davidson, 2012). Domoic acid is also produced by the diatom, *Amphora coffeaeformis* (Lawrence et al., 2011; Lefebvre and Robertson, 2010).

- **Symptoms:** Vomiting, diarrhoea, abdominal pain, confusion, memory loss, seizure, coma and death (Munday and Reeve, 2013).
- **Cases/outbreaks:** The first outbreak of ASP occurred in Canada in 1987 and was related to the consumption of mussels impacted by a bloom of *P. multiseries*. The outbreak affected over 100 cases and four people died. Another outbreak was recorded in 1991 in the state of Washington in the USA, between 11-24 cases were reported following consumption of razor clams (EFSA, 2009b). EFSA (2009b) noted that there were no cases of ASP related illness reported in European countries.
- **Mode of action:** Munday and Reeve (2013) note that there is good evidence that the toxicity of DA relates to "activation of kainic acid and α-amino-3hydroxy-5-methyl-4-isoxazoleproprionic acid receptors in neurones".
- **Toxicity to animals:** Domoic acid exhibits a dose response upon acute administration. There is no evidence of genotoxicity. No data have been published on carcinogenicity or long term toxicity (Munday and Reeve, 2013; Toyofuku, 2006).
- **Maximum permissible level:** Regulation (EC) No 853/2004 states that bivalve molluscs placed on the market for human consumption must not contain ASP at levels exceeding 20 mg/kg of DA.

#### *Yessotoxins (YTX)*

- **The toxins**: Yessotoxins are polyether compounds, more than 90 analogues have been described to date (EFSA, 2008b).
- **The toxin producers:** Primarily produced by the dinoflagellate *Protoceratium reticulatum. Lingulodinium polyedrum* has also been linked to YTX production. Both dinoflagellates have been identified in Scottish waters (Swan and Davidson, 2012).
- **Symptoms:** No symptoms have been recorded in humans (Munday and Reeve, 2013).
- **Cases/outbreaks:** None reported (Toyofuku, 2006).
- **Mode of action:** Unknown (Munday and Reeve, 2013).
- **Toxicity:** Yessotoxins have been shown to be acutely toxic to animals by intra-peritoneal inoculation, however there is no evidence of toxic effects in humans (Lawrence et al., 2011; Munday and Reeve, 2013; Toyofuku, 2006).
- **Maximum permissible level:** Regulation (EC) No 853/2004 (amended by Regulation (EC) No 786/2013) states that bivalve molluscs placed on the market for human consumption must not contain YTXs at levels exceeding 3.75 mg/kg of YTX equivalents. Regulation (EC) No 2074/2005 requires that methods must at least be able to detect the analogues YTX, 45 OH YTX, homo YTX, and 45 OH homo YTX.

### 3.3.2 Mechanism of toxin uptake and sequestration into tissues

As noted previously, most bivalves can effectively retain particles that are 3-4 µm diameter, the size range of particles that can be effectively retained by scallops is slightly larger (5-7 µm). The gills and labial palps are involved in sorting the captured particles, including toxic algae prior to ingestion. Nutritious particles are transported to the mouth for ingestion,

while other particles are rejected in pseudofaeces. Sorting of food for ingestion or rejection is undertaken based on physical and chemical characteristics, such as particle size and the type of carbohydrates present on the algal surface. Once the toxic algae have been ingested, further sorting may occur in the digestive tract with some algal types being selected for intracellular digestion in the digestive diverticula and others being excreted (sometimes intact and live) via the intestine.

For bivalves, toxin contamination occurs primarily through the ingestion of toxic dinoflagellate and diatom cells. However, when algal blooms decline, free toxin is released into the seawater and the uptake of free (dissolved) toxin may also contribute to accumulation by bivalves. Uptake of free toxin may occur through ingestion, but also via absorption across the gills. Recent lab based studies on the uptake of free AZA by mussels (*M. edulis*) have confirmed that mussels can accumulate free AZA to concentrations above the maximum permissible level, indicating that this may be a significant contamination pathway (Jauffrais et al., 2013). The same study noted significant differences in the distribution of the toxin in the anatomical tissues, with most AZA located in the gills following uptake of dissolved AZA, compared with localisation in the digestive tract following uptake of toxic *A. spinosum* cells. The observation that AZA detected in wild and farmed shellfish is predominantly located in the digestive tract, not the gills, led the authors to consider that uptake of free (dissolved) toxin may be less important in bivalve contamination than previously thought (Jauffrais et al., 2013).

### *Toxin localisation*

Marine toxins are predominantly localised in the digestive tissue of bivalves, with generally significantly lower concentrations found in other tissues. There is some suggestion that concentrations of some toxins in non-visceral tissues may increase following prolonged intoxication and/or sometime after the algal bloom has dispersed.

PSP levels in the surf clam (*Spisula solidissima*) from on-shore and off-shore sites were analysed over a two-year period in Maine. It was noted that maximum toxin concentrations were initially detected in the digestive gland, but levels in other tissues increased sometime after a presumed bloom event. PSP concentrations were noted to be highest in the digestive gland > mantle = gill > siphon = foot > adductor muscle (Shumway et al., 1994). Similarly Bricelj (1991) demonstrated that >78% of PSP was localized in the digestive gland of the clam *Mercenaria mercenaria* when exposed to *A. fundyense* and *A. tamarense.*

DSP toxins (OA, DTX1, PTX6) and YTX were injected into the adductor muscle and hepatopancreas of the scallop *Patinopectin yessoensis* and the localisation of toxins studied. It was noted that all toxins remained in the hepatopancreas irrespective of the site of injection. The authors concluded that this justified the analysis of the scallop hepatopancreas in routine monitoring programmes (Suzuki et al., 2005). The distribution of OA and DTX1 was also studied in the mussel *M. galloprovincialis* at different stages of depuration. Results demonstrated that these toxins were largely confined to the visceral tissues with no 'relevant contribution to the toxin burden of non visceral tissues' noted at any stage of the depuration cycle (OA and DTX in non visceral tissues was less than 1% of the total toxin burden) (Blanco et al., 2007).

The concentration of AZA1 and AZA2 were found to be the highest in the hepatopancreas of mussels (*M. edulis)*, with the other tissues containing negligible amounts (between the limit of detection and quantitation). Higher concentration of other analogues (e.g. AZA17), considered to be metabolites of AZA1 and AZA2, were found in the 'other tissues' –

suggesting that these metabolites should be considered in the routine analysis of AZA (Salas et al., 2011). Similarly, Hess et al. (2005) note that the ratio of toxin in the digestive gland in comparison with the whole animal was around five for 28 samples collected over three years in Ireland. It is noted that levels of AZA in the 'other tissues' seems to increase over prolonged periods of intoxication, and that AZA is not exclusively found in the digestive gland (James et al., 2004). AZA was also found to be primarily located in the digestive gland of Queen scallops (>95%), none was detected in the muscle and around 5% was detected in the remaining tissue (Amzil et al., 2008). Similarly, AZA was found to be predominantly located in the hepatopancreas of the King scallop (85%), with less than 6% found in each of the gonad, mantle and gill (Magdalena et al., 2003b).

### *Bivalve feeding response when exposed to harmful algae*

Bivalves display a range of responses when exposed to harmful algae. The physiological and behaviour characteristics displayed include the modification of FR and CR, valve closure and selective rejection or ingestion of toxic dinoflagellates based on size and other chemical and/or physical parameters. The following provides a summary of literature regarding the bivalve feeding response to harmful algae.

Recent research undertaken by Mafra et al. (2010b) suggests the ability of bivalves to selectively ingest and reject harmful algae species based on size. The oyster, *C. virginica,* and mussel, *M. edulis,* were fed the same concentrations of two different clones of *P. multiseries* over a 14-day period. The clones were short (24 µm) and long cell variants (81 µm), the long cell clone produced a higher level of ASP than the short cell clone. Although the longer cell clone of *P. multiseries* was more toxic than the shorter cell clone, the oysters accumulated 50% less DA when presented with the long cell clone compared to the short cell clone. It was noted that the oysters preferentially rejected the long cell clone in its pseudofaeces. In contrast, mussels accumulated higher levels of DA when presented with the long cell clone than when presented with the short cell clone – consistent with the higher toxicity of the long cell variant. The results suggested the ability of the oyster to discriminate harmful algae based on size, whereas mussels did not differentiate and cleared both sized particles from the water with the same efficiency (Mafra et al., 2010b). It may also be possible however, that oysters preferentially rejected the longer cell clone due to the higher level of ASP produced by the long cell compared with the short cell variant.

Shumway and Cucci (1987) carried out experiments to investigate the impact of the PSP-producing dinoflagellate *A. tamarense* on the feeding response of seven species of bivalves. The change in CR of each of the bivalve species was measured in response to the addition of *A. tamarense* cells when compared to a control algal diet. The results indicated a reduction in CR for the eastern oyster, *C. virginica,* and the clam, *M. arenaria*, but not for the mussel, *M. edulis,* or clam, *S. solidissima*. Interestingly, *O. edulis*, the native oyster, showed an increased CR ((Shumway and Cucci, 1987) and reviewed in Bricelj and Shumway (1998)). Similarly, Li et al. (2001) showed that the maximum FR and CR for scallops (*C. nobilis*) was two times higher than that of clams (*R. philippanrium*) when presented with PSP-producing *A. tamarense*.

Clearance rate and PSP accumulation was investigated in five different species of bivalve shellfish that were exposed to toxic and nontoxic strains of *Alexandrium (A. tamarense* and *A. margalefi* respectively) (Contreras et al., 2012). The CRs of the mussel, *P. canaliculus,* and the clam, *Dosinia anus,* were unaffected by the presence of *A. tamarense*, whereas the CR of the clam, *Paphies donacina,* and scallop, *Pecten novaezelandiae,* was reduced in the presence of *A. tamarense.* Consistent with these results, the scallops and clam species

(*P. donacina)* which exhibited reduced CR also showed lower PSP concentrations than mussels and clams (*Donax anus*) that did not alter their CR in response to *A. tamarense* (Contreras et al., 2012).

Bricelj and Shumway (1998) summarised several studies in which the feeding response of bivalves that were fed with mono-specific diets consisting of PSP-producing *Alexandrium* isolates and nontoxic algae were investigated. The mussel *M. edulis* reduced its ingestion rate when fed with a PSP-producing strain of *A. fundyense* when compared with consumption of the nontoxic diatom *Thalassiosira weissflogii* (reviewed in Bricelj and Shumway (1998)[9]). This is consistent with results presented by Bricelj et al. (1990) which showed a 48% reduction in CR for mussels presented with *A. fundyense* compared with mussels presented with a nontoxic control diet. Similarly, the CR of the oyster, *C. gigas,* declined when presented with PSP-producing strains of *A. minutum* and *A. tamarense*, compared with oysters presented with nontoxic *Scrippsilella trochoidea* (reviewed in Bricelj and Shumway (1998)).

A recent investigation undertaken on the uptake of AZAs by common mussels (*M. edulis*) also demonstrated that mussels reduce CR and FR when exposed to the AZA producing dinoflagellate *A. spinosum.* An initial study showed that AZA uptake by mussels was rapid with levels exceeding the maximum permissible limit within six hours, however after this time accumulation slowed significantly, additionally, observations of increased mussel mortalities were made (Jauffrais et al., 2012b). This led to further investigations to evaluate the feeding response of *M. edulis* to *A. spinosum: A. spinosum* was found to have a significant negative effect on the mussels. The results indicated that high concentrations of *A. spinosum* resulted in a reduction in the mussel CR and FR, by factors of six and three respectively, an increased production of pseudofaeces was also noted (Jauffrais et al., 2012a).

Consistent with these studies, which suggest interspecies differences in response to harmful algae, Hegaret et al. (2007) investigated the feeding responses of five species of bivalves that were exposed to three species of harmful algae: the PSP-producing dinoflagellate *A. fundyense, Prorocentrum minimum*, and the potential brevetoxin producing algae *Heterosigma akashiwo*. The bivalves investigated included the scallop *Argopecten irradians*, *C. virginica*, *M. edulis*, *M. arenaria* and the northern quahog *M. mercenaria*. The results demonstrated that the CR "varied appreciably between different bivalve/alga pairs". Additionally, the authors noted that the oysters closed in response to all three harmful algal types presented. Scallops presented with *A. fundyense* closed at the start of the experiments, but soon re-opened, whereas mussels stayed open for the entire experiment in response to all algal types. It was considered that valve closure, as displayed by the oysters, is a mechanism employed by some bivalves to limit tissue damage related to potential ingestion of harmful algae (Hegaret et al., 2007).

Other researchers have also noted that some shellfish species close their valves in response to harmful algae before slowly re-opening. Shumway and Cucci (1987) demonstrated that *C. virginica* showed initial valve closure in response to *A. tamarense,* followed by gradual re-opening. In the same study mussels (*M. edulis*), collected from a local region in Maine USA, did not display valve closure in response to *A. tamarense,* whereas mussels sourced from

[9]Raw data obtained from Lee, J. The kinetics of PSP toxin transfer from the toxic dinoflagellate Alexandrium spp. to two bivalve mollusc species, *Mytilus edulis* and *Mercenaria mercenaria*. M.S. thesis, State University of New York, Stony Brook, NY. 168 p. (1993) (thesis not reviewed for this report).

Spain, which were thought to have no previous exposure to *A. tamarense*, exhibited at least partial shell closure. The authors proposed that mussels that are periodically exposed to toxin-producing algae may have an adaptation mechanism that allows them to continue to feed normally during subsequent exposures (Shumway and Cucci, 1987).

Like *M. edulis* sourced from Maine, research undertaken on the feeding response of the greenshell mussel *P. canaliculus* when presented with toxic and nontoxic strains of *A. tamarense* demonstrated normal valve opening responses and faecal production (Marsden and Shumway, 1992). The previous history of exposure of the mussels to *A. tamarense* was unknown.

Similar to the findings of Shumway and Cucci (1987) and Hegaret et al. (2007) regarding the valve closure response of the oyster *C. virginica*, Wildish et al. (1998) found that the Pacific oyster, *C. gigas,* also demonstrated a 'stop/start' clearance behaviour when exposed to toxic and nontoxic strains of *A. tamarense* and *A. fundyense.* Significantly fewer oysters initiated feeding when presented with *A. tamarense* and *A. fundyense* compared to those offered the nontoxic *Isochrysis* sp. (Tahitian strain).

### 3.3.3 Comparison of toxin accumulation and elimination between bivalve species

There have been a multitude of separate studies that have evaluated toxin uptake and loss in a single bivalve species. However, relative accumulation and elimination of toxins by different species is difficult to evaluate using the results from separate studies that have been undertaken independently under different environmental/ambient conditions. Therefore, the following sections provide an overview of studies conducted where concurrent monitoring of multiple bivalve species have been undertaken for each regulated toxin group. Tables 3.5 to 3.8 provide a summary of the maximum concentration of toxin (for each regulated toxin group) reported from various bivalve species during simultaneous sampling in the field, and during laboratory experiments.

Some caution needs to be taken when comparing toxicity maxima between species and considering the appropriateness of a particular bivalve (e.g. mussels) as an indicator, as toxin accumulation and elimination rates during a bloom are likely to vary both within and between species (e.g. comparative levels between species are likely to change as a bloom progresses). Therefore, the maximal values presented may not always provide a complete picture of whether the indicator species would have given adequate forewarning of toxicity in other species. Additionally, some bias may be introduced when species occupy different habitats, and because some species are sampled with a much higher frequency than others.

Studies that contribute significantly to the conclusions of this review have been subjected to a critical appraisal process to assist the reader to evaluate the usefulness of the findings of key publications. The process used for appraisal is detailed in the methodology (Section 2) and the findings of the critical appraisal are included in Appendix 1.

#### *Comparative accumulation of paralytic shellfish poisons (PSP)*

In response to the discovery of PSP in French shellfish, a laboratory study was undertaken to investigate the comparative accumulation and elimination of PSPs in four different species of commercial bivalve shellfish (Lassus et al., 1989). The study involved feeding *M. edulis, C. gigas, P. maximus* and *R. philippanarum* the same quantities of a PSP-producing strain of *A. tamarense*, followed by a decontamination stage. Shellfish were analysed by both the mouse bioassay and HPLC throughout the experiment. Low contamination rates of oysters and clams were noted after 15 and 16 days of feeding respectively: the maximum

concentrations recorded in both species were approximately 1000 µg/kg (estimated from Figure 2 of Lassus et al. (1989)). Much higher PSP concentrations were detected in scallops and mussels after only five days exposure. The maximum concentrations recorded in scallops and mussels were 27000 and 11000 µg/kg respectively; notably the scallop accumulated higher levels of PSP than the mussel (Table 3.5). Two-phase decontamination curves were observed for both scallops and mussels, with a rapid decrease followed by a slow decline. Toxin profiles were also evaluated in the study and noted to differ between the four species, with rapid elimination of some analogues (e.g. GTX3, GTX8) observed in comparison to others (e.g. GTX2) (Lassus et al., 1989). Lassus et al. (1989) also noted that in September 1988 there was a toxic bloom of an *Alexandrium* species (species not noted) in northern Brittany. Oysters were reported to have a maximum concentration of 2550 µg/kg, while mussels had a maximum level of 4000 µg/kg (species not noted).

A comparison of PSP concentrations between the scallop *P. yessoensis*, *M. edulis* and the oyster *C. gigas* was undertaken in Japan in 1978 when these shellfish species were collected from Ofunato Bay (Shizu Station), and also in 1979 when shellfish were collected from Kesennuma and several other stations in the Miyagi prefecture (Sekiguchi et al., 2001). The plankton species responsible for PSP in the shellfish was shown to be *A. tamarense*. Concurrent PSP testing of scallops and mussels throughout an *A. tamarense* bloom in Ofunato Bay demonstrated that the toxicity of scallops always exceeded that of *M. edulis* by 1.5 to eight times – during both the toxin uptake and elimination phase of contamination (Figure 3.2). The oyster *C. gigas* also contained PSPs, but at much lower concentrations than both mussels and scallops. Two species of scallops, *P. yessoensis* and *C. nippoensis akazara,* sampled from four separate sites in 1979 also always showed higher levels of PSP than concurrently sampled *M. edulis* and *C. gigas* (Table 3.5). Elimination of PSPs was investigated in *P. yessoensis* and *M. edulis* that had been harvested from Ofunato and then placed in tanks for five months. PSPs in mussels decreased quickly to non-detectable concentrations after one month, whereas PSPs in scallops declined rapidly initially and then were retained for a long period of time with toxin still detectable after five months. Similarly, Sekiguchi et al. (2001) undertook feeding experiments in which mussels (*M. galloprovincialis*), oysters (*C. gigas)*, clams (*R. philippinarum)* and scallops (*P. yessoensis)* were fed with PSP-producing *A. tamarense* and then subjected to a 12 day depuration period in tanks. Toxicity in mussels, oysters and clams was observed to drop rapidly, but scallops retained high levels of PSP throughout the depuration period (Sekiguchi et al., 2001).

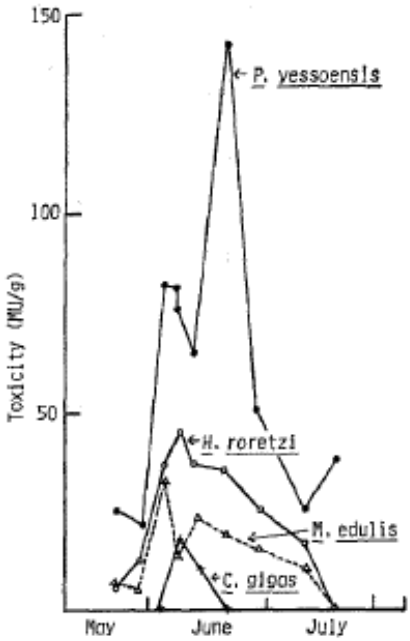


**Figure 3.2:** Comparison of PSP concentrations (mouse units/g) in the scallop *P. yessoensis,* mussel *M. edulis*, oyster *C. gigas* and the tunicate *Holocythia roretzi* during an *Alexandrium tamarense* bloom in Ofunato, Japan, 1978. Figure reproduced from Oshima et al. (1982).

In tank accumulation studies were undertaken on *M. edulis* and the clam *R. philippinarum* (Lassus et al., 1994). The PSP producing dinoflagellate *A. minutum* was fed to both species, however the clams received a concentration of *A. minutum* that was approximately seven-fold higher than the mussels. Despite the higher dose provided to the clams, both species reached approximately the same concentration after 15 days of exposure (approximately 1800 µg/kg). The results indicated that the uptake of PSP by *R. philippinarum* is slower than for the mussel *M. edulis*. Following the accumulation phase of the experiments, a depuration period was undertaken in which nontoxic food (*Tetraselmis*) was provided. The results indicated that mussels took around five days for levels to reduce below the maximum permissible limit (800 µg/kg) and clams took around eight days, despite containing very similar starting concentrations (Lassus et al., 1994).

Similar to the results of Lassus et al. (1989), it was noted that during an algal bloom in 1972 in the USA, that clams and oysters were non-toxic (species not noted), but co-occurring scallops (*A.* irradians) and mussels (*M. edulis*) contained toxin levels of 20000 and 26000 µg/kg (reviewed in Bricelj (1991). Further, a review by Bricelj and Shumway (1998) notes that "it is well documented that *M. edulis* generally becomes two to four times more toxic than neighbouring or co-neighbouring (clam) *M. arenaria*."

Uptake studies were performed on the Pacific oyster (*C. gigas*) and the King scallop (*P. maximus)* which were exposed to a constant concentration of a PSP producing strain of *A. minutum* for 9 – 14 days (five individual experiments of varying length were performed) (Bougrier et al., 2003). Data presented suggests that the maximum PSP concentration accumulated in oysters (900 µg/kg) was higher than that of scallops (450 µg/kg) (extrapolated from data presented in Figure 4 of Bougrier et al. (2003)).

A range of bivalve species were sampled from two sites in Masinloc Bay, Philippines during blooms of the toxic dinoflagellate *P. bahamense* var *compressum* in 2000 and 2001. At Site 1 the thorny oyster, *Spondylus squamus,* was shown to have very high toxin levels in both years in comparison to simultaneously sampled species of penshell and rocky oyster (*Atrina vexillum* and *Chama iostoma*) (Table 3.5). At Site 2, PSP concentrations in the mussel *Perna viridis)* were greater than 500 µg/kg, whereas blood cockles and the common oyster (*Anadara antiquata* and *Crassostrea* sp.*)* were found to have no significant toxicity (Montojo et al., 2006) (Table 3.5).

As noted previously, the CR of the eastern oyster *C. virginica* was inhibited when exposed to toxin producing cells, in contrast the CR of the native oyster, *O. edulis,* increased. Further, Bricelj and Shumway (1998) summarise data from the USA, which suggests that *O. edulis* can reach higher toxicities than *C. virginica* (although it should be noted that these data are not from concurrent monitoring i.e. same site, same time). The authors also quote unpublished data (J. Hurst) which suggested that *O. edulis* becomes toxic before *M. edulis* in the same area (Bricelj and Shumway, 1998).

Table 3.5 presents data obtained from the above-published studies in which concurrent monitoring of PSP in multiple bivalve species was undertaken. It can be seen from this summary that mussels have consistently accumulated higher levels of PSPs than oysters and clams that were concurrently sampled. Scallops also appear to be able to accumulate relatively high levels of PSP; in a laboratory study PSP levels in the King scallop were reported to exceed those of mussels (Lassus et al., 1989). A separate study involving concurrent field monitoring of *M. edulis* and two species of scallops revealed consistently higher concentrations of PSP in scallops compared with mussels during both the uptake and depuration phase of contamination (Oshima et al., 1982). It should be noted in the case of scallops that PSP is predominantly located in the digestive gland which is commonly/routinely removed prior to human consumption.

**Table 3.5:** Maximum PSP concentrations reported from various bivalve species during concurrent sampling in the field, and during laboratory experiments. Maximum permissible level = 800 µg/kg. ND = not detected. [a]PSP concentrations reported by Oshima et al (1982) were expressed in mouse units/g. One mouse unit (MU) has been determined to have a value of 0.18 µg STX.2HCl, this value has been used to convert the reported values from MU/g to µg/kg.

| Species | Shellfish | PSP level µg/kg | Location | Date | Source |
|---|---|---|---|---|---|
| *M. edulis* | Common mussel | 26000 | New England, USA | 1972 | Reviewed in Bricelj (1991) |
| *A. irradians* | Scallop | 20000 | | | |
| Not noted | Oyster | ND | | | |
| Not noted | Clam | ND | | | |
| *P. yessoensis* | Scallop | [a]1980, 3420 | Site 17, Kesennuma, Japan | 1979 | Oshima et al. (1982) |
| *Chlamys nippoensis akazara* | Scallop | 3960, 2880 | | | |
| *M. edulis* | Common mussel | 1350, 1008 | | | |
| *C. gigas* | Pacific oyster | ND, ND | | | |
| *P. yessoensis* | Scallop | [a]2160, 3060 | Site 18, Kesennuma, Japan | 1979 | Oshima et al. (1982) |
| *Chlamys nippoensis akazara* | Scallop | 486, 378 | | | |
| *M. edulis* | Common mussel | 702, ND | | | |
| *C. gigas* | Pacific oyster | ND, ND | | | |
| *P. yessoensis* | Scallop | [a]4140, 4140 | Site 21, Motoyoshi, Japan | 1979 | Oshima et al. (1982) |
| *Chlamys nippoensis akazara* | Scallop | 3600, 8280 | | | |
| *M. edulis* | Common mussel | 1386, 2520 | | | |
| *C. gigas* | Pacific oyster | ND, ND | | | |
| *P. yessoensis* | Scallop | [a]8640 | Site 24, Okatsu, Japan | 1979 | Oshima et al. (1982) |
| *M. edulis* | Common mussel | 5220 | | | |
| *C. gigas* | Pacific oyster | 324 | | | |
| *P. yessoensis* | Scallop | [a]15660, 13550 | Site 25, Okatsu, Japan | 1979 | Oshima et al. (1982) |
| *M. edulis* | Common mussel | 6300, 2880 | | | |
| *C. gigas* | Pacific oyster | 432, ND | | | |
| *P. yessoensis* | Scallop | [a]6120 | Site 26, Onagawa, Japan | 1979 | Oshima et al. (1982) |
| *Chlamys nippoensis akazara* | Scallop | 8280, 4320 | | | |
| *M. edulis* | Common mussel | 2880, 450 | | | |
| *C. gigas* | Pacific oyster | ND | | | |
| Not noted | Mussels | 4000 | Brittany, France | 1988 | Lassus et al. (1989) |
| Not noted | Oysters | 2550 | | | |
| *P. maximus* | King scallop | 27000 | Lab study | 1989 | Lassus et al. (1989). Oyster and clam data estimated from Figure 2. |
| *M. edulis* | Common mussel | 11000 | | | |
| *C. gigas* | Pacific oyster | 1000 | | | |
| *R. philippinarum* | Manila clam | 1000 | | | |
| *C. gigas* | Pacific oyster | 900 | Lab study | 1998 – 2001 | Data estimated from Figure 4 of Bougrier et al. (2003) |
| *P. maximus* | King scallop | 450 | | | |
| *S. squamosus* | Thorny oyster | 1174 | Masinloc Bay, Philippines | 2000 & 2001 | Montojo et al. (2006) |
| | | 2916 | | | |
| *C. iostoma* | Rocky oyster | 133 | | | |
| | | 190 | | | |
| *P. viridis* | Mussel | 500 | Masinloc Bay, Philippines | 2000 | Montojo et al. (2006) |
| *Crassostrea* sp. | Common oyster | ND | | | |
| *A. antiquata* | Blood cockle | ND | | | |

### *Comparative accumulation of diarrhetic shellfish poisons (DSP)*

In 2011, three human illnesses occurred which were related to DSP in mussels (*M. edulis)* from the United States Pacific North West. In response to this, monitoring for DSP commenced in Washington State in 2012. Between May and November 2012 mussels (*M. edulis*), oysters (*C. gigas)* and clams (*Leukoma staminea*) were concurrently collected and analysed for DSP from each of two different sites. The results demonstrated that the mussels consistently contained higher levels of DSP than the oysters and clams. The highest

concentration of DSP occurred in mussels (1030 µg/kg), compared with a maximum of 50 µg/kg and 7 µg/kg recorded in oysters and clams respectively (Table 3.6) (Trainer et al., 2013). *D. acuminata* was the main DSP producing species observed.

Similar observations were made by Lindegarth et al. (2009) in field experiments which involved suspending the native oyster *O. edulis* and the common mussel *M. edulis* in cages at a depth of 3 m in the water column for four weeks during a *D. acuta* bloom. The concentration of OA group toxins (including OA, DTX1, DTX2 and the esters of these compounds) in the native oyster was 10 – 50 fold lower than in mussels, despite the co-location of both species at the same place in the water column. Concentrations in mussels exceeded the MPL for DSP by 10-fold, whereas oysters did not reach the limit. The authors suggested that the results indicate that "*O. edulis* is a low risk species for DST contamination". Following the accumulation of the DSP toxins, oysters and mussels were subjected to a seven-week depuration period in the laboratory. While differences in elimination rates of the various congeners were noted, there were no major differences in elimination rate between the two species (Lindegarth et al., 2009).

Lee et al. (2011) collected mussels (*M. galloprovincialis*) and oysters (*C. gigas*) from six and seven stations respectively along the south coast of Korea between 2007 and 2009 and analysed the samples for DSPs (OA, DTX1 and PTX2) and yessotoxins. DTX2 and DTX3 were not included in the analysis. Mussels and oysters were concurrently collected on the same day from several of the stations, at depths of 3 m from hanging rope culture. It was noted that at Station 9 between June and August 2007 DSP concentrations in mussels (hepatopancreas) ranged between 114.9 to 142.5 µg/kg, whereas oysters collected at the same time had lower DSP concentrations in the hepatopancreas tissue, between 1.3 and 11.3 µg/kg. Consistent with this, mussels collected from Station 3 were noted to have DSP levels exceeding 10 µg/kg on five occasions in 2007/2008, whereas oysters at the same site were below the limit of quantitation. The maximum concentration of OA, DTX1 and PTX1 detected in whole oysters was 3, 12 and 14 µg/kg respectively, whereas the maximum concentration of these toxins in whole mussels was 39, 107 and 20 µg/kg respectively (Lee et al., 2011).

In 2003 and 2004 in the Thermakios Gulf (Eastern Mediterranean) a variety of bivalve shellfish were collected during two consecutive blooms of *D. acuminata.* The samples were analysed for free OA (the analysis did not include DTXs or esters). The results showed that the mussel *M. galloprovincialis* contained the highest level of OA (3222.2 µg/kg). The mussel *Modiolus barbatus* was found to contain a maximum of 647.8 µg/kg, whereas scallops (*Flexopecten proteus* and *Chlamys varia*) and clams (*Venus verrucosa)* contained much lower levels with maxima of 148.9, 80.4 and 37.9 µg/kg respectively. The authors concluded that *M. galloprovincialis* is the most appropriate indicator for DSP in the Thermakios Gulf (Reizopoulou et al., 2008).

Similar results were obtained in an earlier study in which the accumulation of DSPs, including the acyl esters, was compared in mussels, *M. galloprovinvialis*, and scallops, *P. yessoensis*, harvested from the same site in Japan*.* Mussels and scallops were both harvested from a depth of 20 m during a bloom of *D. fortii*. OA was not detected in any samples. DTX1 and DTX3 were found to be significantly higher in the mussels than the scallops (Table 3.6). DTX1 was found to be the dominant analogue in mussels, whereas DTX3 was the dominant analogue in scallops (Suzuki and Mitsuya, 2001).

Duinker et al. (2006) undertook research in which the common mussel, native oyster and King scallop were exposed to blooms of *Dinophysis* spp and samples collected over a six-week period during the accumulation phase of contamination. Large interspecies variations in toxin content were noted, with mussels containing 10 – 20 fold higher concentrations of DSP than oysters or scallops (Duinker et al., 2006).

A study was undertaken in 2003 in Portugal on the kinetics of OA, DTX and PTX contamination of the blue mussel (*M. galloprovincialis*) and the cockle (*C. edule*) during a bloom of *D. acuta.* The study involved the monitoring of DSP in cockles and mussels from the Ria de Aveiro; the mussel and cockle sites were separated by four kilometres. Higher levels of OA and DTX2 were observed in mussels than cockles, this generally reflected the higher toxicity of plankton at the mussel site. Interestingly, higher levels of PTX-2SA were detected in cockles than in the mussels. The results showed that cockles and mussels accumulate and eliminate the various toxin analogues differentially – confirming both inter species variation in uptake and elimination of toxins, and intra species variation in the uptake and elimination of different toxin analogues (Vale, 2004a).

During the event, contaminated mussels and cockles were harvested and then placed into tanks of recirculating seawater and fed a diet composed of a mixture of non-toxic algae. OA, DTXs (including the acylated forms) and PTXs were then monitored in the cockles and the mussels. The elimination of all PTXs (including PTX2 and PTX2-sa) was very rapid in mussels and slower in cockles: the elimination rate was two to three times faster for mussels than cockles. In contrast, cockles were found to eliminate DTX2 faster than mussels (mussels retained DTX2 for longer) (Vale, 2004a). Following four days of decontamination in tanks, the mussels had eliminated 41% of the total OA (including free and ester forms), whereas the cockles had eliminated around 86%. In a separate similar study which investigated the elimination of DSPs by the clam species *Donax trunculus* and *S. solida*, 33% and 59% of the total OA had been eliminated from each species respectively after four days. Accordingly, the authors suggest that the "elimination of DTXs by *D. trunculus* is slower than that by *M. galloprovincialis"* (Vale, 2006).

Table 3.6 shows a summary of data presented in the literature from studies in which concurrent monitoring of DSP in multiple bivalve species was undertaken. In summary, the results of studies to date suggest that various species of mussels accumulate higher levels of the DSP toxins than other bivalve species concurrently sampled from the same area (including various species of oysters, clams and scallops). While some bivalves, such as the cockle, *C. edule*, eliminate DSP (not including PTX) at faster rates than mussels, there is evidence that other bivalve species, such as the clam, *D. trunculus*, may retain DSP toxins for longer than mussels species.

**Table 3.6:** Maximum DSP (OA, DTX and PTX) concentrations reported from various bivalve species during concurrent sampling in the field, and during laboratory experiments. Maximum permissible level = 160 µg/kg. ND = not detected. Tr = Trace.

| Species | Shellfish | DSP level (µg/kg) | DSP analogues included | Location | Date | Source |
|---|---|---|---|---|---|---|
| *M. edulis* | Mussels | 1030 | OA, DTX1, 2 & 3 | Washington State, USA | 2012 | Trainer et al. (2013) |
| *C. gigas* | Pacific oysters | 50 | | | | |
| *L. staminea* | Clams | 7 | | | | |
| *M. galloprovincialis* | Mussels | 114.9 – 142.5 | OA, DTX1, PTX1 | Korea | 2007 | Lee et al. (2011) |
| *C. gigas* | Pacific oysters | 1.3 – 11.3 | | | | |
| *M. galloprovincialis* | Mussels | 3222.2 | OA | Thermakios Gulf, Eastern Mediterranean | 2003 & 2004 | Reizopoulou et al. (2008) |
| *M. barbatus* | Mussels | 647.8 | | | | |
| *F. proteus* | Scallop | 148.9 | | | | |
| *C. varia* | Scallop | 80.4 | | | | |
| *V. verrucosa* | Clam | 37.9 | | | | |
| *M. galloprovincialis* | Mussels | 11000/2300 | DTX1/DTX3 | Japan | 2 July 1996 | Suzuki and Mitsuya (2001) |
| *P. yessoensis* | Scallops | Tr/1000 | | | | |
| *M. galloprovincialis* | Mussels | 6200/3200 | DTX1/DTX3 | Japan | 8 July 1996 | Suzuki and Mitsuya (2001) |
| *P. yessoensis* | Scallops | Tr/1600 | | | | |

### *Comparative accumulation of azaspiracid*

A review undertaken in 2008 summarised detections of azaspiracid in shellfish and crustaceans and demonstrated reasonably widespread contamination, with positive findings in many European countries, northern Africa and Canada (Furey et al., 2003; James et al., 2004; Taleb et al., 2006; Twiner et al., 2008). Despite the increasing detection of AZA worldwide, very few studies involving the concurrent monitoring of AZAs in multiple species of shellfish have been reported; therefore this section focuses more on maximum levels detected in shellfish monitoring programmes to date.

Between 1999 and 2001, five species of shellfish were harvested from a range of shellfish production areas throughout Ireland and monitored for AZA. *M. edulis* accumulated the highest level of AZA during the study (4200 µg/kg) and oysters (*C. gigas*) were also found to contain high levels (maximum reported 2450 µg/kg). Scallops (*P. maximus)*, cockles (*C. edule)* and clams (*R. philippinarum*) contained lower levels, but the maximum recorded for each species was above the maximum permissible level for AZA (Table 3.7). A large proportion (92%) of the mussels tested (n=800) were positive for AZA, as were 67% of the oysters (n=150), 75% of the scallops (n=40) and 15% of the clams (n=20) and cockles (n=40). Levels of AZA in oysters collected from the same site as mussels were noted to be up to five times more toxic than mussels on occasion. Oysters from County Donegal (Site 7) contained 2450 µg/kg AZA compared with 1800 µg/kg in mussels collected on the same day from the same site. Thus the authors suggested that "oysters can be efficient accumulators of AZA" whereas clams and cockles were suggested to constitute a lower risk due to reduced accumulation (Furey et al., 2003). Supporting the conclusions of Furey et al. (2003) regarding efficient uptake of AZA by oysters, data reviewed in Lawrence et al. (2011) shows oysters and mussels (species not specified) apparently concurrently sampled on three separate occasions contained approximately equal concentrations of AZA (Table 3.7).

In contrast, a summary of AZA detection in a range of shellfish species sourced from commercial production areas in Ireland over the summer months in 2001 (July to October) showed that *M. edulis* (1500 µg/kg; n=452) accumulated higher levels than *C. gigas (*420 µg/kg; n=306), *O. edulis* (40 µg/kg; n=31), *E. siliqua* (80 µg/kg; n=26) and *P. maximus* (320 µg/kg hepatopancreas; n=25)(Hess et al., 2003) (Table 3.7). It should be recognised however, that it is not specified if these shellfish (or some of) were sourced from the same

production areas at the same times, and were therefore exposed to the same sources of toxin.

Salas et al. (2011) summarised monitoring data for AZAs in shellfish cultured in Ireland between 2003 and 2010, and displayed the maximum concentration detected in each species. It is suggested that concentrations above the maximum permissible level had only been detected in blue mussels (*M. edulis*) and Pacific oysters (*C. gigas*) in Ireland over this period. The maximum level noted for mussels and oysters was 8970 µg/kg and 310 µg/kg respectively (Table 3.7) (Salas et al., 2011). A recent review also notes that AZAs are present in much lower concentrations in species other than mussels, including razors of the same species (*E. arcuatus* and *E. siliqua)* as those harvested in Scotland (Tillmann et al., 2014). Data obtained from the literature prior to 2003 however, shows a maximum level of 2450 µg/kg recorded for oysters, and that scallops, cockles and clams had exceeded the regulatory limit (Furey et al., 2003; Hess et al., 2003).

King and Queen scallops (*P. maximus* and *A. opercularis*) from France have also been found to contain AZAs at elevated levels (320 and 274 µg/kg respectively) (reviewed in Jauffrais et al. (2012b). The detection of AZA in Queen scallops occurred on the Brittany coast in France in 2006 (Amzil et al., 2008), similarly the King scallops were also from Brittany (Magdalena et al., 2003a). An investigation into the food safety risks related to AZA in the King scallop in Ireland was undertaken; scallops from four sites were collected between 1999 and 2001 and tested for AZA. The maximum level detected in scallop hepatopancreas during the study was 1940 µg/kg (Magdalena et al., 2003b). One outbreak of human illness (20 – 30 illnesses) has been attributed to the consumption of King scallops in September 1998 in France. The scallops were reported to have originated from Bantry Bay, Ireland; levels of toxin associated with the implicated scallops were not reported (Twiner et al., 2008). Of note, James et al. (2004) referred to an illness incident in France in September 1998 involving the same number of cases, but attribute the incident to mussel consumption.

AZA1 and AZA2 were identified in the following species harvested from the Portugese coast in 2006: *M. edulis, C. edule, Venerupis senegalensis, Ruditapes decussatus, Solen marginatus* and *Crassostrea* spp in 2006. While AZA was detected in these species, levels in whole flesh were extremely low and ranged from 1.6 – 6.1 µg/kg (Vale et al., 2008a). This finding followed a previous study in 2002 in which 300 samples from Portugal were analysed for AZA and no positives were detected (Vale, 2004b).

Table 3.7 presents a summary of data from published studies in which concurrent monitoring of AZA, or maximum levels of AZA, are reported for multiple bivalve species. It can be seen from the data obtained from general monitoring programmes (such as those noted in the second half of the table entitled 'maximum AZA concentration reported'), that mussels have accumulated significantly higher levels of AZA than other species that are routinely monitored. There is some evidence that at times oysters may contain higher levels of AZA than mussels (Table 3.7, concurrent sampling), however it is unclear at what point in the intoxication process the concurrent samples were collected and if the oysters contained more AZA at the start of the event, during the event, or through the toxin depuration phase.

**Table 3.7:** Maximum AZA concentrations reported for various bivalve species during concurrent sampling and routine monitoring programmes. Maximum permissible level = 160 µg/kg. LoQ = Limit of Quantitation. NS = not specified. n = number of samples analysed.

| Species | Shellfish | n | Maximum AZA level µg/kg | Location | Date | Source |
|---|---|---|---|---|---|---|
| **Concurrent Sampling** | | | | | | |
| NS<br>NS | Oyster<br>Mussel | NS<br>NS | 700<br>700 | County Cork, Ireland | Nov 1998 | Reviewed in Lawrence et al. (2011) |
| NS<br>NS | Oyster<br>Mussel | NS<br>NS | 300<br>100 | County Donegal, Ireland | Nov 1999 | Reviewed in Lawrence et al. (2011) |
| NS<br>NS | Oyster<br>Mussel | NS<br>NS | 200<br>100 | County Cork, Ireland | Feb 2000 | Reviewed in Lawrence et al. (2011) |
| *C. gigas*<br>*M. edulis* | Oyster<br>Mussel | 1<br>1 | 2450<br>1800 | County Donegal, Ireland | 1999 - 2001 | Furey et al. (2003) |
| ***Maximum AZA concentration reported in routine monitoring programmes*** | | | | | | |
| *M. edulis* | Common mussel | 800 | 4200 | Ireland | 1999 - 2001 | Furey et al. (2003) |
| *C. gigas* | Pacific oyster | 150 | 2450 | | | |
| *T. philippinarum* | Manila clam | 40 | 610 | | | |
| *P. maximus* | King scallop | 40 | 400 | | | |
| *C. edule* | Cockle | 20 | 200 | | | |
| *M. edulis* | Common mussel | 452 | 1500 | Ireland | 2001 | Hess et al. (2003) |
| *C. gigas* | Pacific oyster | 306 | 420 | | | |
| *P. maximus* | King scallop (hepatopancreas) | 25 | 320 | | | |
| *E. siliqua* | Razor | 26 | 80 | | | |
| *O. edulis* | Native oyster | 31 | 40 | | | |
| *M. edulis* | Common mussel | NS | 8970 | Ireland | 2003 - 2010 | Salas et al. (2011) |
| *C. gigas* | Pacific oyster | NS | 310 | | | |
| *S. solida* | Surf clam | NS | 150 | | | |
| *T. philippinarum* | Manila clam | NS | 100 | | | |
| *C. edule* | Cockle | NS | 80 | | | |
| *O. edulis* | Native oyster | NS | 70 | | | |
| *E. arcuatus* | Razor | NS | 50 | | | |
| *G. glycymeris* | Dog cockle | NS | 10 | | | |
| *T. semidescussatus* | Clam | NS | 10 | | | |
| *E. siliqua* | Razor | NS | <10 | | | |
| *Haliotis discus hannai* | Abalone | NS | <LoQ | | | |
| *P. vulgata* | Common limpet | NS | <LoQ | | | |
| *V. senegalensis* | Pullet carpet shell | NS | <LoQ | | | |
| *V. verrucosa* | Venus clam | NS | <LoQ | | | |

### *Comparative accumulation of amnesic shellfish poisons (ASP)*

Following the illness outbreak related to ASP in mussels in Canada (1987), investigations were undertaken in Ireland to determine if DA was present in Irish shellfish (James et al., 2005). The study involved the collection and analysis of four different species of bivalves: mussels (*M. edulis*), oysters (*C. gigas*), razor clams (*E. siliqua*), and King scallops (*P. maximus*). The samples were collected over a six-month period from sites around the coast of Ireland. DA was detected at trace levels in razors (n=14), mussels (n=97) and oysters (n=60). Only 2% of the oyster and mussel samples collected were positive. In contrast, 89% of the scallop samples (whole tissue tested) (n=175) were positive and 55% exceeded the regulatory limit of 20 mg/kg. The authors noted that DA levels in mussels and scallops were

different even when samples came from the same production area: “mussels cultivated in Bantry Bay using ropes suspended in deep waters, consistently did not contain detectable levels of DA, whilst scallops, cultivated along the shore line, had very high levels of toxin (up to 2270 mg DA/kg hepatopancreas).” (Table 3.8) (James et al., 2005).

Following the initial detection of DA in French shellfish in 1998, further monitoring was undertaken in 1999 and 2000, with samples of four bivalve species (mussels, cockles and two clam species) collected in each year between April and August, as *Pseudo-nitzschia* blooms over this period were considered more likely (Amzil et al., 2001). ASP levels in 1999 were found to be very low, however elevated levels were detected in 2000, particularly on the Western Brittany coast. Two species were sampled in this area, the clam *D. trunculus* and the mussel *M. edulis/galloprovincialis*. *D. trunculus* (53 mg/kg) had a higher maximum concentration of ASP than *M. edulis galloprovincialis* (31 mg/kg). Toxin accumulation was related to the presence of *P. multiseries* and *P. pseudodelicatissima* (Amzil et al., 2001).

Concurrent feeding experiments were undertaken on the oyster *C. virginica* and mussel *M. edulis;* the shellfish were fed the same concentrations of two different ASP producing clones of *P. multiseries* (short and long cell clones) over a 14 day period (Mafra et al., 2010b). *C. virginica* accumulated 3 – 7.5 times less DA than mussels when fed with the short cell clone. The result was consistent with the reduced clearance rates observed for oysters (7.4 to 8.5 times lower than mussels). ASP levels were 70 times lower in oysters than mussels when presented with the long cell clone. The maximum concentration of DA accumulated by oysters was 44 mg/kg, whereas the maximum observed in mussels was 320 mg/kg. The authors noted that these results were consistent with data reported from Canadian Food Inspection Agency in 2002 during a bloom of *P. seriata,* in which mussels had maximum DA levels of 200 mg/kg compared with only 0.9 mg/kg in oysters (Mafra et al., 2010b).

Similar results were reported in a separate study, also by Mafra et al. (2010a). This involved the simultaneous ‘in tank’ exposure of mussels (*M. edulis)* and oysters (*C. virginica*) to *P. multiseries* for four and seven days respectively. Mussels were found to accumulate 8 – 17 fold higher concentrations of DA than oysters, and reached maxima of 460 mg/kg and 78.6 mg/kg respectively. Toxin levels were also monitored following an uptake period of two days and subsequent depuration phase of 21 days. Nontoxic algae (*Isochrysis galbana* and *Pavlova pinguis*) were fed to the mussels and oysters during the depuration phase. Domoic acid elimination rates of mussels were found to be two to four fold higher than oysters of the same size (Mafra et al., 2010a).

Table 3.8 shows a summary of published data from studies in which concurrent monitoring of ASP in multiple bivalve species was undertaken. Mussels accumulated higher levels of DA than the eastern oyster. In contrast, the King scallop has been found to accumulate higher levels of DA than mussels, as has the clam *D. trunculus*. In relation to the scallop and clam data presented in Table 3.8, it is unclear at what stage in the progression of a bloom the samples were collected, therefore some caution needs to be taken when considering whether mussels are an appropriate indicator – as it may be possible that mussels contained higher levels of DA than scallops and clams at a different stage in the bloom.

**Table 3.8:** Maximum ASP (domoic acid) concentrations reported from various bivalve species during concurrent sampling in the field, and during laboratory experiments. Maximum permissible level = 20 mg/kg. ND = not detected. Table modified from Table 1 presented in Mafra et al. (2009).

| Species | Shellfish | ASP (mg/kg) | Location | Date | Source |
|---|---|---|---|---|---|
| *M. edulis*<br>*C. virginica* | Common mussel<br>Eastern oyster | 460<br>78.6 | Lab study | 2007 | Mafra et al. (2010a) |
| *M. edulis*<br>*C. virginica* | Common mussel<br>Eastern oyster | 320<br>44 | Lab study | 2005 - 2006 | Mafra et al. (2010b) |
| *M. edulis*<br>*C. virginica* | Common mussel<br>Eastern oyster | 200<br>0.9 | Canada | 2002 | Canadian Food Inspection Agency |
| *P. maximus*<br>*M. edulis* | King scallop<br>Common mussel | 2270<br>ND | Bantry Bay, Ireland | 2000 | James et al. (2005) |
| *D. trunculus*<br>*M. edulis galloprovincialis* | Clam<br>Mussel | 53<br>31 | Brittany coast. France | 2000 | Amzil et al. (2001) |

### *Comparative accumulation of yessotoxin*

Yessotoxin has not been conclusively linked to human illness via the consumption of shellfish. Perhaps consistent with the lack of 'cause and effect', there has been less international focus on determining patterns of uptake and elimination of yessotoxin in different shellfish species (when compared to the other toxin groups), with most research focus being placed on identification of new toxin analogues, toxicological studies and the development and validation of new methodologies. While it is apparent from the literature that YTX has been widely detected in various species of mussels and scallops, few studies have investigated the relative accumulation of YTX between species. Nonetheless, several comparative studies on uptake of YTX have been undertaken.

During 2005, Portuguese shellfish were screened for YTX during the summer and autumn months. Samples were collected from the Aveiro and Formosa lagoons, and at the Algarve (off-shore). In the lagoon sites it was noted that YTXs were detected in decreasing concentrations in the following species: *M. galloprovincialis* > *C. edule* > *R. decussatus* (concentrations not noted). *Crassostrea* spp. were also tested but no YTX was detected. At the off-shore site higher YTX concentrations were detected in *S. solida* than in *Donax* spp. At Aveiro lagoon increases in YTX were associated with the presence of *Protoceratium* spp. and *Gonyaulax spinifera,* and at the off-shore site *L. polyedrum* was present (Vale et al., 2008b; Gomes et al., 2006).

In 2007, mussel, oyster and clam (species not noted) samples collected from the French Mediterranean coastline showed positive results for YTX and its analogues. The maximum concentration reported in mussels was 10 mg/kg, whereas only trace levels were reported in oysters and clams (Amzil et al., 2008). Consistent with this report which suggests higher accumulation of YTX by mussels than oysters, Pacific oyster (*C. gigas*) samples (n= 242) and mussel (*M. galloprovincialis)* samples (n=214) were collected from 7 sampling stations along the south coast of Korea between 2007 and 2009 and tested for YTX. The maximum concentration of YTX recorded in whole oyster tissue was 4 µg/kg (0.004 mg/kg), compared with a maximum of 8 µg/kg (0.008 mg/kg) in whole mussel tissue (Lee et al., 2011).

In contrast to these field-based observations, yessotoxin was reported to accumulate and detoxify at faster rates in the oyster *C. gigas* compared with the mussel *M. edulis* in a lab-based study (Röder et al., 2011). The study involved feeding oysters and mussels YTX producing *P. reticulatum* over a 16-day period, followed by a four day 'rest' period and a 16-day detoxification period. Unfortunately, the study does not note whether the amount of

*P. reticulatum* available to each species of bivalve was standardised using the bivalve body (meat) weight, which may confound interpretation of this data.

### 3.3.4 Possible reasons for interspecies differences in accumulation

Toxin accumulation in bivalves is related to feeding processes that regulate toxin intake (e.g. filtration, clearance, ingestion and digestion) and detoxification processes that govern toxin elimination. Thus, there are many factors that potentially impact the efficiency of bivalves to concentrate a particular toxin type. The following provides a summary of the main parameters that appear to influence toxin accumulation.

#### *Interspecies differences in clearance, filtration and ingestion rates*

There are significant interspecies differences in the clearance and filtration rates (CR and FR respectively) of bivalve shellfish that inhabit the same environment and are therefore exposed to the same environmental conditions. These interspecies differences in CR have been confirmed to result in differential uptake of harmful algae by bivalves e.g. Li et al. (2001) demonstrated that scallops have a CR that is two times greater than clams when fed with *A. tamarense.* They noted that this provides some explanation for field observations that scallops are found to contain higher levels of PSP than clams located in the same area.

The concentration of algal cells in the seawater (or seston concentration) has also been clearly demonstrated to result in changes in the CR and FR of bivalves, as has seawater temperature and salinity. Different bivalve species have different conditions under which CR and FR are optimal, and sub-optimal conditions can lead to CR inhibition. Reduction in CR is documented to correlate with reduced toxin uptake by some bivalve species.

For example, it was demonstrated that the CR of oysters (*C. virginica)* when presented with *P. multiseries* was 7.4 – 8.5 times lower than the CR of *M. edulis.* The reduced CR of oysters was directly related to a reduction in the uptake of DA in oysters compared with mussels (3 – 7.5 times lower DA in oysters) (Mafra et al., 2010b). It was noted that the CR of the oysters increased with temperature from 1 – 18°C, likewise the accumulation of DA also increased with temperature. The authors noted that "differential toxin uptake by these two co-occurring bivalves both in the field and in the laboratory is strongly affected by inter-specific differences in clearance rate, which can be exacerbated at low temperatures" (Mafra et al., 2010b).

Sekiguchi et al. (2001) undertook in-tank feeding experiments in which scallops (*P. yessoensis*), mussels (*M. galloprovincialis)*, oysters (*C. gigas*) and clams (*R. philippinarum)* were fed on PSP-producing *A. tamarense* cells over an eight-day period. For bivalves presented with the same number of cells, PSP levels were very similar, thus oysters, mussels and clams accumulated comparable concentrations of PSP as they were fed on the same number of *A. tamarense* cells during the experiments. However, scallops that were presented with three times more cells than other bivalves accumulated significantly higher levels of PSP. Based on these results the authors propose that inter-specific differences in toxin accumulation may be greatly affected by ingestion rate, with higher ingestion rates leading to higher levels of toxicity within animals (Sekiguchi et al., 2001).

Seston concentration and salinity have been clearly shown to impact the CR of bivalves in laboratory-based studies. However, there are few field studies where toxin concentration of bivalves has been measured alongside seston concentration, or toxin concentration measured with salinity; thus there is limited information available to directly correlate these

factors with toxin accumulation. Further information on bivalve CR responses to seston concentration, salinity and temperature can be found in Section 3.1.2.

### *Modification of bivalve clearance rate in response to the presence of harmful algae*

Bivalves display a range of responses when presented with toxic algae, including valve closure and selective rejection or ingestion of toxic dinoflagellates based on size and other chemical and/or physical parameters. Section 3.3.3 summarises currently available information on these responses, which vary widely between bivalve species. For example, Hegaret et al. (2007) found significant differences in CR between different bivalve and harmful algae pairs (five bivalve species and three types of harmful algae were investigated), and in response to this finding noted "generalisations about feeding responses of bivalves to harmful algae cannot easily be made".

The robustness of generalisations regarding CR and FR of bivalves is compromised by the large variations observed between and within species in response to a range of factors (e.g. food quality and availability, temperature and salinity). Nonetheless, generally studies to date suggest that species of oysters (particularly *C. virginica* and *C. gigas)* and clams frequently exhibit valve closure and reduced CR and/or FR in response to the presence of harmful algae. In contrast, most (but not all) studies report that mussels and scallops don't exhibit significant or prolonged valve closure and relatively smaller reductions in CR or FR when compared to other bivalve species.

These general responses of bivalves to harmful algae are reasonably consistent with the between species differences in toxin accumulation that are noted in Section 3.3.4, which suggest that mussels generally contain higher levels of toxins than oysters and clams, and that scallops may contain higher levels of ASP and PSP than mussels at times. Additionally, several studies have directly correlated changes in CR with toxin accumulation rates e.g. Contreras et al. (2012); Jauffrais et al. (2012a); Mafra et al. (2010b). Many studies have noted a reduction in CR when toxic algae are presented to some bivalves, and considered that this provides an explanation for higher levels of accumulation in some species compared to others (Section 3.3.3). Given the foregoing, it is considered that variation in feeding responses to harmful algae plays a significant role in the observed differences in toxin accumulation between bivalve species.

It has been suggested that the observed differences in feeding response of bivalves to harmful algae may be explained by the relative sensitivity of bivalves to the negative impacts of harmful algae. A review undertaken by Bricelj and Shumway (1998) noted that the "feeding response [of bivalves] to toxic cells is also a good indicator of toxin sensitivity and the potential for toxin accumulation in bivalves". Accordingly, it was suggested that there is a reasonably good correlation between the nerve sensitivity of bivalves to PSP and toxin accumulation, with species that show low or no nerve sensitivity readily feeding on toxic cells (e.g. *M. edulis*) and those which show high sensitivity (e.g. *C. gigas*) displaying physiological (e.g. reduced CR) and behavioural traits (e.g. valve closure) to avoid exposure to harmful algae (Bricelj and Shumway, 1998).

### *Food selection processes*

Previously it was noted that the bivalve feeding process involves a series of particle selection steps, namely: (1) sorting of particles on the gills; (2) selection on the labial palps; and (3) preferential adsorption of some algae types following ingestion.

Firstly, with regard to particle sorting on the gills, bivalves can discriminate food particles on the basis of size and this has been demonstrated to result in differences in toxin accumulation between bivalve species. It was shown that oysters (*C. virginica*) preferentially ingested short cells of *P. multiseries* compared to long cells, and accumulated 50% less DA when presented with longer cells. Mussels presented with both the short and long cells showed no size discrimination and accumulated significant levels of DA regardless of the algal size. The results demonstrated selective ingestion by the oyster based on the size of the toxic algae. The authors noted that the size selection relates to physical limitations in the structural arrangements of the oyster gills as compared to mussel gills. This may provide some further explanation as to why oysters generally accumulate very low levels of toxins (with the potential exception of AZA) compared to mussels and scallops. It was further suggested that scallops have much larger filament apertures than oysters and mussels and thus selective rejection of *P. multiseries* by the scallop based on size is unlikely (Mafra et al., 2010b).

Secondly, with respect to selection on the gills and labial palps (prior to ingestion), recent research is noted that shows an interaction between carbohydrates on the algal cell surface and lectins within the mucus which covers the pallial organs. The authors suggest that this is a common mechanism for particle selection (Espinosa et al., 2010a; Espinosa et al., 2009, 2010b). This mechanism could account for observations of preferential ingestion of particular algal types by certain bivalve species, as shown in previous studies (Bricelj, 1991; Shumway et al., 1985).

Lastly, food that is ingested by bivalves is also subject to selective processes in the stomach – with unwanted food particles passing to the intestine and excreted in the faeces, and other particles passing to the digestive diverticula for intra cellular digestion. Differences in adsorption efficiency, along with pre-ingestive selection processes, are also highly likely to contribute to observed differences in toxin accumulation between bivalve species.

### *Exposure to toxic cells*

Harmful algae blooms can be very localised in the marine environment (i.e. 'patchy'), and occupy distinct niches in order to exploit the resources they need for survival (e.g. they stratify at particular depths in the water column to optimise light, nutrients etc.). Commercially produced bivalve shellfish in Scotland occupy different marine habitats (e.g. some inhabit the seafloor, others are grown subtidally on long lines, and others are intertidal) (Table 3.4). Algal bloom patchiness, together with the noted differences in marine habitat that shellfish occupy, can lead to significant variation in shellfish exposure across small geographic spaces, both within and between species.

Several studies were summarised in a review undertaken by Bricelj and Shumway (1998) that demonstrate the significant spatial variation in shellfish toxicity that can occur due to non-uniform distribution of algal cells in the seawater:

- The PSP concentration of subtidal mussels placed nearshore was reported to be two orders of magnitude lower than mussels suspended in 15 metres of water 300 – 600 m off-shore;
- PSP concentrations of off-shore mussels were two to five times more toxic when suspended at the surface than the bottom; and
- PSP accumulation in scallops was noted to vary markedly with water column depth over 30 m (reviewed in Bricelj and Shumway (1998)).

While differences in exposure to toxic algae are likely to contribute to the observed interspecies variation in accumulation of toxins, this does not explain all between species differences in toxin accumulation. For example, Lindegarth et al. (2009) undertook a study in which the native oyster (*O. edulis*) and common mussels (*M. edulis),* which typically do not occupy the same habitat, were suspended in cages in the same location within the water column. Mussels accumulated significantly higher (10-50 fold) concentrations of DSP (OA group toxins and PTXs) than co-located oysters.

### *History of toxin exposure*

It has been suggested that bivalves that are periodically exposed to algal blooms acclimate and more readily accumulate toxins than those which have not been exposed previously Supporting this suggestion, one study has noted that mussels (*M. edulis)* with no history of prior exposure exhibited the valve closure response when exposed to *A. tamarense,* in contrast with those with a prior history of exposure to *A. tamarense* which did not close their valves (Shumway and Cucci, 1987). Additionally, it was noted that mussels (*M. edulis)* accumulated 50% less PSP during an *Alexandrium* bloom when compared to mussels with a long term history of exposure (reviewed in Bricelj and Shumway (1998)). It has been considered that mussels with a history of toxin exposure may adapt to the presence of toxic algae to enable them to continue normal feeding activities.

It has become increasingly popular in recent times for shellfish to be sourced at a range of sizes and be relayed into production areas for on-growing until they reach commercial size. It is possible that shellfish sourced externally for on-growing may have a different toxin history to those that have been raised locally, and this could introduce the potential for intra- and interspecies differences in toxin accumulation – if history of toxin exposure plays a significant role in accumulation.

### *Toxin elimination*

PSP elimination rates for a variety of bivalve shellfish were extensively reviewed in Bricelj and Shumway (1998) and the data demonstrates significant interspecies variation in elimination. Consistent with this, common mussels (*M. edulis*) depurated PSP faster than manila clams (five days vs. eight days) (Lassus et al., 1994), and were also shown to eliminate PSPs more rapidly than the scallop *P. yessoensis* (Oshima et al., 1982). Similarly, scallops (*P. yessoensis)* were found to retain PSPs more efficiently than mussels, oysters and clams in an in-tank depuration study (Sekiguchi et al., 2001).

This review also highlights several examples in which the elimination rates of DSP and PTXs differs considerably between bivalve species, for example:

- The elimination of all PTXs (including PTX2 and PTX2-sa) was shown to be two to three times faster for mussels than cockles (Vale, 2004a);
- The depuration of DTXs was found to be faster in cockles than mussels, with 86% and 41% of the total OA present eliminated after four days in each species respectively (Vale, 2004a); and
- The elimination of DTXs by the mussel (*M. galloprovincialis*) was considered to be faster than for the clam (*D. trunculus*), with 41% and 33% eliminated after four days respectively (Vale, 2006).

For ASP, elimination rates of mussels (*M. edulis)* were found to be two to four fold higher than oysters (*C. virginica)* of the same size (Mafra et al., 2010a).

The reasons for variation in toxin elimination rates both within and between bivalve species is generally not well understood. However, it seems likely that this relates to a range of physiological and biochemical factors, such as: the rate of defaecation and excretion of toxins, the conversion of the toxins from one toxin analogue to another, and the degradation of the toxins to nontoxic compounds within the bivalve. Some evidence exists to support the roles of defaecation and interconversion of toxin analogues in the detoxification process (Bricelj and Shumway, 1998).

### 3.3.5 Summary of potential issues associated with the use of indicators

Schwacke et al. (2013) established a series of criteria to assist in evaluating the effectiveness of marine indicator species. The findings of the literature review are thus considered in the context of the two major criteria proposed (Section 3.2).

***Criterion 1 'Sensitivity for bioaccumulation': an indicator species should be highly sensitive to concentration of the toxin or contaminant, and be more sensitive than the other shellfish species of interest.***

The previous sections demonstrate that when concurrent monitoring occurs, mussels generally accumulate higher levels of toxins than most other species that co-occur in the same area; however there are some exceptions.

- For PSP and ASP, results from laboratory and field studies suggest higher toxin concentrations may periodically accumulate in scallops (*P. maximus, P. yessoensis* and *C. nipponensis akazara*) than mussels (*M. edulis)* (James et al., 2005; Lassus et al., 1989; Oshima et al., 1982).
- For ASP, one field study reported higher concentrations present in clams (*D. trunculus)* than mussels (*M. edulis galloprovincialis)* (Amzil et al., 2001).
- For AZA, there is some suggestion that oysters (*C. gigas)* are efficient accumulators and may contain higher concentrations of AZA than mussels (*M. edulis)* in certain situations (Furey et al., 2003; Lawrence et al., 2011).
- For YTX, one lab-based study notes that oysters (*C. gigas)* accumulate YTX at a faster rate than mussels (*M. edulis)*. However, the study did not describe if levels of YTX presented to oysters and mussels were standardised on a body weight basis, which may affect the interpretation of the results considerably (Roder et al., 2011). In contrast, field studies on YTX suggest higher levels are present in mussels than oysters, clams and cockles that were co-occurring in the production area (Amzil et al., 2008; Lee et al., 2011).
- For DSP, all studies that were reviewed demonstrated higher concentrations of toxin in mussels compared with oysters, scallops and clams that were sampled concurrently.

An evaluation of the robustness of the publications noted above has been undertaken (Appendix 1).

These results suggest that for PSP and ASP caution should be applied when considering the use of mussels as an indicator species for scallops and clams. Similarly, for AZA caution should be applied when considering the use of mussels as an indicator species for oysters.

With respect to toxin elimination, this review highlights several examples in which the depuration rate of toxins was found to differ significantly between species. Notably, the

elimination rate of DSP from two species of clams is suggested to be slower than the elimination rate of DSP from mussels.

A significant number of studies have examined the detoxification of PSP from bivalves and were comprehensively summarised in Bricelj and Shumway (1998). These demonstrate that the time to reduce levels below the maximum permissible level (800 µg/kg) varied markedly between species. The authors also calculated the percentage loss of toxin per day (elimination rate) for a range of species, which enabled them to classify species into two groups: fast and slow detoxifiers. Notably, the mussel *M. edulis*, Pacific and native oysters, and the scallop *P. maximus* were classified as fast detoxifiers. Whereas, the cockle *C. edule* was considered a slow detoxifier.

Given the significant interspecies variation in toxin elimination, and the general observation that mussels are rapid detoxifiers, it is recommended that consideration be given to collecting concurrent monitoring data for multiple species produced in the same area during the toxin elimination phase, to support risk management decisions regarding which indicator species may be appropriate immediately following toxin events.

***Criterion 2 'Appropriate distribution': Indicator species should have a distribution that overlaps with the other shellfish species of interest.***

Table 3.4 provides an overview of the preferred habitat of commercial shellfish stocks in Scotland. It can be seen from this summary that some species, such as mussels, are grown suspended in the water column from long lines, others are grown on the seafloor (e.g. scallops), and others inhabit the intertidal zone (e.g. oysters and clams). Those that inhabit the intertidal zone, such as oysters, have shorter immersion and feeding times than those that are grown subtidally – thus oysters may be exposed to harmful algae for shorter time periods than the subtidal species. This may also contribute to the generally lower levels of toxicity observed for oysters compared to mussels.

With respect to scallops, however, which are cultured on the seafloor in several areas in Scotland, it is noteworthy that benthic dinoflagellates and diatoms may pose a higher risk to scallops than suspended shellfish such as mussels. Additionally scallops may be at greater risk of contamination following the termination of blooms when algal cells drop to the benthos and become available for consumption. This may account for observed differences in toxin concentrations between bivalve species, and the periodically elevated levels of ASP and PSP in scallops in comparison to mussels.

## 3.4 Chemical contaminants

### 3.4.1 The contaminants

A broad range of chemical contaminants can arise in the marine environment from both anthropogenic and biogenic origins. As discussed in previous sections, bivalve shellfish are filter feeders and this mechanism of feeding can lead to the accumulation of contaminants that may be present in the surrounding seawater. If significant quantities of certain contaminants are accumulated, people who consume the shellfish may become ill. Generally illness related to the intake of chemical contaminants is caused by long-term exposure to the contaminants in the environment and/or through the on-going consumption of a range of contaminated food types (i.e. acute illness related to one food type, such as shellfish, is less likely). In order to protect human health, shellfish are routinely monitored for a variety of contaminants which are specified in EU law, including: (a) lead (Pb), cadmium (Cd) and mercury (Hg); (b) polycyclic aromatic hydrocarbons (PAHs); and (c) dioxins and dioxin-like

PCBs (DL-PCBs). The following sections provide a brief overview of the defining characteristics of the regulated contaminants noted above. Information has primarily been collated from a number of recent comprehensive reviews undertaken by the FAO/WHO and EFSA.

### *Lead (Pb)*

- **The contaminant:** Lead belongs to group 4A of the periodic table, has an atomic number of 82, and an atomic mass of 207.2 g/mol. The main oxidation states of Pb are +2 and +4. Lead occurs in both organic and inorganic forms, with the latter dominating in the environment.
- **The source:** Lead is naturally occurring in the environment, but also occurs to a greater extent as a result of anthropogenic activities such as mining, smelting and battery manufacture. Non dietary exposure to Pb is thought to pose a lower risk than dietary exposure for the general EU population (EFSA, 2010a).
- **Human health impacts:** Chronic toxicity is considered the highest concern. Lead is neurotoxic and has been found to affect central information processing (including short term verbal memory). The developing brain is considered more susceptible to Pb than the mature brain; there is a relationship between reduced IQ and elevated Pb levels in children up to seven years of age. There is also an association between chronic kidney disease, elevated blood pressure and Pb blood concentrations in adults. (Reviewed in EFSA (2010a)). There have been many historical and recent studies that have found that people exposed to elevated Pb concentrations have increased mortality rates associated with renal and/or cardiovascular disease. Additionally, many studies have found associations between lower dose Pb exposure and non-fatal symptoms such as neurotoxicity and cardiovascular effects (EFSA, 2010a)
- **Mode of action:** Lead is absorbed through the gastrointestinal tract and then transported via the blood to the soft tissues (e.g. liver, kidney) and to bone. Pb accumulates with increasing age. The main target organ for Pb is the central nervous system. Pb has an affinity for thiol groups and other organic receptors in proteins, this has been attributed to many of the toxic effects of Pb. Another major factor in the toxicity of Pb is that it can substitute for calcium and zinc (EFSA, 2010a).
- **Toxicity in animals:** Rodent and primate studies have demonstrated that long-term low-level exposure leads to neurotoxicity, especially learning deficits in developing animals. There is also evidence that Pb induces increased blood pressure and nephrotoxicity, and that it may be genotoxic to experimental animals. Lead is well documented to cause tumours in rodents and was classified as “probably carcinogenic to humans (group 2A) in 2006” (EFSA, 2010a).
- **Maximum permissible level:** Regulation (EC) 1881/2006 specifies the maximum permissible level for Pb in bivalves (amended by Regulation (EC) 620/2008) = 1.5 mg/kg.

### *Cadmium (Cd)*

- **The contaminant:** Cadmium is a transition metal occupying group IIB of the periodic table, is stable in the Cd(II) (aq) ion and has an ionic radius of 109 pm (Dobson et al., 2014; EFSA, 2009c).
- **The source:** Cadmium occurs naturally in the environment as a result of volcanic activity and erosion of rocks/sediments. Anthropogenic sources also contribute to Cd in the marine environment, including activities such as: wastewater release, mining, waste

incineration, application of fertilisers and subsequent run off etc. (EFSA, 2009c, 2012a; Gueguen et al., 2011).

- **Human health impacts:** A large number of studies have been published on the human health effects of Cd, much of this information has been summarised in recent reviews (EFSA, 2009c, 2012a). Acute Cd intoxication is rare, however in cases in which high levels of Cd are present in food, humans have acute gastrointestinal symptoms. The main concern for the general population relates to chronic exposure. Effects of long-term exposure to elevated levels of Cd include renal damage (Cd accumulates in the kidneys), bone demineralisation, delayed foetal growth, reduced fertility, and increased risk of cancer (EFSA, 2009c). Human population studies undertaken in Japan and Belgium confirmed that Cd is a renal toxicant. Similarly, Cd has been confirmed to be a carcinogen. Recent epidemiological studies also found a positive association between decreased bone mineral density and dietary Cd intake (reviewed in Dobson et al. (2014)).
- **Mode of action:** At elevated levels Cd induces breakages in DNA and genome instability, it also inhibits DNA repair which may result in its genotoxic effects. Cadmium is also reported to affect gene transcription and translation, which may contribute to carcinogenicity. Cadmium may induce anaemia through direct damage to the proximal renal tubular cells. (EFSA, 2009c).
- **Toxicity in animals:** EFSA (2009c) note toxicity to animals has been reviewed by many agencies and that the target organs (kidney and bones) and toxicokinetics following oral administration is "roughly similar" between species, however adsorption in rodents appears to be lower than humans. See section above on 'symptoms' for the main features of toxicity.
- **Maximum permissible level:** Regulation (EC) 1881/2006 specifies the maximum permissible level for Cd in bivalves (amended by Regulation (EC) 620/2008) = 1.0 mg/kg.

### *Mercury (Hg)*

- **The contaminant:** Three forms of Hg are found in the environment: (a) elemental Hg; (b) inorganic Hg; and (c) organo-metallic compounds, of which methylmercury (MeHg) is the most common form. The main forms of Hg in seawater are elemental and organic Hg (e.g. MeHg). The methylation of Hg is facilitated by iron-reducing and sulphate-reducing bacteria (EFSA, 2012c).
- **The source:** Mercury arises in the environment from both natural and anthropogenic sources. Naturally occurring mercury is released into the environment during volcanic eruptions, degassing of the earth's crust and from water evaporation. Anthropogenic emissions are related to coal mining, burning and other industrial activities (EFSA, 2012c).
- **Human health impacts:** Neurological health risks associated with mercury have been documented since the late 1800s (early studies mainly related to laboratory exposure). Acute exposure can result in neurological damage e.g. mental retardation, cerebral palsy, deafness, blindness and dysarthria if exposed *in utero*, and motor impairment in exposed adults. Several significant poisoning events have occurred. Notably, in the 1950s in Minimata, Japan, neurodevelopmental toxicity was observed in a population that was exposed to environmental sources of Hg (through release of wastewater) via consumption of contaminated fish. Thousands of cases of poisoning were reported (Harada, 1995; reviewed in EFSA (2012c). Chronic, low-dose long-term exposure to MeHg is associated with poor performance in neurobiological tests that measure attention, language, memory and fine-motor function. There is some evidence that MeHg affects

the cardiovascular system. The developing foetus is particularly susceptible to mercury. (EFSA, 2012c; Gueguen et al., 2011).

- **Mode of action:** Methylmercury is quickly absorbed in the human gut and enters the brain. It accumulates in the brain where it exerts its effects over the individual's lifetime. Interactions between MeHg and cellular biomolecules likely result in cytoskeletal alterations, oxidative stress and disturbances in calcium homeostasis which can cause toxicity effects (EFSA, 2012c).
- **Toxicity in animals:** Exposure of lab animals via the oral route to Hg in the form of methylmercuric chloride at relevant doses, results in damage to the kidneys, stomach and intestine, changes in blood pressure and heart rate, and adverse impacts on sperm and male reproductive organs. Additional studies report increases in embryonic lethality, decrease in foetal body weight and teratogenicity in rats (EFSA, 2012c).
- **Maximum permissible level:** Regulation (EC) 1881/2006 specifies the maximum permissible level for Hg in bivalves (amended by Regulation (EC) 620/2008) = 0.5 mg/kg.

### *Polycyclic aromatic hydrocarbons (PAHs)*

- **The contaminant**: The polycyclic aromatic hydrocarbons (PAHs) comprise a large group of organic compounds (which consist of solely hydrogen and carbon atoms). They each have two or more fused aromatic rings. The PAHs generally occur in mixtures of compounds, sometimes with hundreds of different PAHs present (EFSA, 2008c).
- **The source:** PAHs are formed through incomplete combustion and decomposition of organic matter at high temperatures (pyrolysis), and from natural and anthropogenic sources e.g.
  - Industrial energy production
  - Use of incinerators
  - Heating via fires, gas or oil
  - Production of aluminium, steel and iron
  - Petroleum catalytic cracking
  - Gas and diesel powered engines
  - Production of asphalt, coal tar and coke
  - Sewage
  - Forest fires
  - Volcanoes (reviewed in EFSA (2008c); Gueguen et al. (2011); Webster et al. (2010)).
- **Human health impacts:** Data on human oral exposure to PAHs is very limited. FAO/WHO (2006) suggest that there are no direct studies that evaluate the association between PAH intake and cancer risk. Similarly, EFSA (2008c) note major data limitations regarding human observations.
- **Mode of action:** PAHs may be absorbed through the gastrointestinal tract. PAHs are oxidised to form primary and secondary metabolites that are more polar and water-soluble. Increased water solubility may lead to increased elimination/excretion of PAHs. PAH metabolites may bind to DNA and proteins. The interaction of the PAH metabolites with DNA may result in damage to the DNA and carcinogenesis (FAO/WHO, 2006).
- **Toxicity in animals:** In 2006 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) summarised available LD50 data, which was predominantly generated for mice and rats via the oral and intraperitoneal routes. The data suggests that PAHs have low-moderate acute toxicity (FAO/WHO, 2006). Benzo(a)pyrene is considered to be

carcinogenic when administered by the oral route, with tumours of the gastrointestinal tract, liver, lungs and mammary glands of mice and rats reported. Limited oral testing has been undertaken regarding the carcinogenicity of other PAHs (EFSA, 2008c). JECFA commented that PAH metabolites have been observed binding to DNA (predominantly to guanine and adenine), which may account for carcinogenicity (FAO/WHO, 2006). FAO/WHO (2006) also note "15 of the individual PAHs show a clear genotoxic action in standard assays *in vitro* and *in vivo".*

- **Maximum permissible level:** Regulation (EC) 1881/2006 specifies maximum permissible levels for PAHs in bivalves (amended by Regulation (EC) 835/2011):
    - Benzo(a)pyrene: 5.0 µg/kg
    - Sum of benzo(a)pyrene, benzanthacene, benzo(b)fluoranthene and chrysene: 30 µg/kg.

***Dioxins and dioxin-like polychlorinated biphenyls (DL-PCBs):***

- **The contaminants**: 'Dioxins' is the term used to describe a large group of polychlorinated, planar aromatic compounds. The group has similar structures and chemical and physical properties and consists of around 75 dibenzo-*p*-dioxins (PCDDs) and 135 dibenzofurans (PCDFs). They are lipophilic and bind to sediments and organic matter. Dioxin like-PCBs are organochlorine compounds that are synthesised through the chlorination of biphenyl. The DL-PCBs consist of 12 congeners which show similar toxicological properties to dioxins (EFSA, 2012b).

- **The source:** Dioxins are formed as an unwanted by-product of a number of industrial processes, including metallurgy, waste incineration and burning such as combustion engines, wildfire, wood burning etc. Dioxin like-PCBs are synthesised due to important properties such as non-flammability, stability, high boiling point etc. They have widespread uses such as: transformer and condenser oils, paint, plastics and sealants. Dioxin like-PCBs were produced in large quantities up until the 1980s but production has reduced due to the implementation of restrictions in some countries, similarly, emissions of dioxins have decreased since the 1980s. Both dioxins and DL-PCBs are broadly distributed in the environment due to leakages and improper disposal. (EFSA, 2012b; Gueguen et al., 2011).

- **Human health impacts:** Symptoms of high exposure include dermal and ocular effects. Cognitive impairments in children and infants have been associated with exposure of the growing foetus to PCBs through the consumption of contaminated fish. There are also increased concerns for breastfed infants, as they may be exposed to higher intakes. Populations in Yusho, Japan (1700 victims) and Yu-Cheng, Taiwan (2000 victims) were exposed to elevated levels of PCBs and dioxins in rice oil (1968 and 1978 respectively) and were observed to have cognitive impairment, increased tumour incidence and neurological, endocrine, hepatotoxic and immunotoxic effects (reviewed in Anonymous (2000); van Larebeke et al. (2001)). There have been several accidents reported in more recent years relating to the contamination of animal feeds and foods, including the contamination of 500 tons of animal feed with 50 kg of PCBs and 1 g of dioxins in Belgium in 1999. Some reports estimate that cancers in the general population will be between 40 and 8000 as a result of the incident (van Larebeke et al., 2001).

- **Mode of action:** The mechanism of action of dioxins and PCBs is thought to relate to the interaction of dioxins and DL-PCBs with an intracellular protein called the *Ah* receptor. The *Ah* receptor is a transcription enhancer which interacts with other proteins such as heat shock proteins, kinases etc. It is present in most vertebrates, which generally seem

to exhibit similar sensitivity to dioxins and DL-PCBs as humans (Birnbaum, 1994; Van den Berg et al., 2006).

- **Toxicity in animals:** A variety of effects are reported in laboratory animals, including endometriosis, cognitive effects, immunotoxic effects and developmental reproductive effects. Some dioxins have been demonstrated to be carcinogenic in several species (Anonymous, 2000).
- **Maximum permissible level:** Regulation (EC) 1881/2006 specifies maximum permissible levels for dioxins and DL-PCBs in bivalves (amended by Regulation (EC) 1259/2011).
    - Sum of dioxins: 3.5 pg/g
    - Sum of dioxins and DL-PCBs: 6.5 pg/g

### 3.4.2 Mechanism of contaminant uptake and sequestration into tissues

As noted in the previous section, contaminants arise in the marine environment through natural processes, such as volcanic activity and the erosion of rocks/sediments, and through a range of anthropogenic activities. The main forms of Hg present in seawater are elemental and organic Hg (e.g. MeHg). MeHg is suggested to comprise around 5% of total Hg present in estuarine and oceanic waters (EFSA, 2012c). Cd can exist in seawater in free soluble form or complexed with organic and non-organic substances. The complexed forms are relatively non-motile, however soluble (free) Cd can migrate in the water column and EFSA (2009c) note "cadmium is most readily absorbed by aquatic organisms in its free form, $Cd^{2+}$". Pb occurs in ionic form, in organic complexes, associated with colloidal particles and attached to clay or organisms (e.g. plankton) within the marine environment. Similar to Cd, Pb is considered to be highly mobile and bioavailable in the free form. Low molecular weight (MW) PAHs are reasonably water soluble, whereas high MW PAHs are relatively insoluble (hydrophobic). The insoluble PAHs may bind to suspended particulates such as plankton and sediments (Baumard et al., 1999; EFSA, 2008c; Webster et al., 2010). Dioxins and DL-PCBs are relatively insoluble in water and mainly associated with sediments and other particulate matter in the water columns.

Given the foregoing, the contaminants of regulatory concern exist in two formats in the marine environment: soluble forms that are free in the water column, and insoluble forms that are attached to particulate matter such as clay, silt, microalgae etc. Phytoplankton may become contaminated through the adherence of chemical compounds onto the surface of the cell, and sometimes contaminants diffuse into the cell and exist in the cell cytoplasm (Gueguen et al., 2011). Accordingly, accumulation of contaminants by bivalves occurs in two ways: (1) soluble contaminants are accumulated directly across the gill surface; and (2) insoluble contaminants that are complexed with particulate matter are bioaccumulated through the process of bivalve filter feeding.

Consistent with these two modes of accumulation, many studies have found significant concentrations of metal ions (including Cd, Hg and Pb) associated with the bivalve gill and digestive gland tissue (Cunningham and Tripp, 1975; Dimitriadis, 2003; Domouhtsidou and Dimitriadis, 2000; Soto et al., 1996; Soto et al., 2002). Soto et al. (1996) used autometallography techniques to demonstrate that Cd was localised in the abfrontal epithelial cells of the gill of the mussel *M. galloprovincialis*. Black silver deposits (which indicates the presence of Cd) were also observed around the apical edge of the stomach epithelial cells and digestive ducts, supporting the notion that the digestive gland is the major site of accumulation. They also noted that the mantle epithelium showed the presence of black silver deposits, assimilation or excretion through the mantle seems probable given it is directly in contact with seawater. Connective tissue directly below the

mantle showed the presence of Cd within brown cells. Brown cells may play a role in transportation of metals from the target organs for excretion via the stomach and intestine and facilitate detoxification of the mussel (Soto et al., 1996).

A similar study was undertaken to investigate the distribution of Hg and Pb in *M. galloprovincialis.* Mussels were exposed to Hg and Pb (0.1 mg/L) over 30 and 60 days and autometallography and x-ray microanalysis was used to analyse the sub-cellular localisation within the mussels. The study demonstrated accumulation in the epithelial and sub-epithelial cells of the labial palps, in the lysosomes of the digestive cells and in the abfrontal region of the gills (Dimitriadis, 2003)

Collectively these results clearly demonstrate significant metal accumulation in both digestive and gill tissues. An earlier study investigating the uptake, tissue distribution and elimination of Hg in the oyster *C. virginica* demonstrated that the solubility state of the metal in the surrounding water effects the concentrations that are assimilated in each tissue type (Cunningham and Tripp, 1975). The oysters were exposed for three days to Hg (in two different forms: $HgCl_2$ and $CH_3HgCl$), either directly in artificial seawater, or via the addition of the Hg-labelled diatom *Phaeodactylum tricornutum*. When the oysters were exposed to Hg that was added directly to the seawater the Hg concentrations in tissues were (in decreasing order): gill > digestive system > mantle > gonad > muscle. When oysters were presented with diatoms labelled with Hg, the Hg concentrations in tissues were digestive system > gill > mantle > gonad > muscle (Cunningham and Tripp, 1975). The study demonstrated that soluble metals are predominately assimilated via the gills, whereas particulate bound metals primarily accumulate in the digestive gland through feeding.

As noted previously, low MW PAHs are soluble in the water column, whereas high MW PAHs are relatively insoluble but bind to particular matter in the water column. Research undertaken on mussels in France (*M. edulis* and *M. galloprovincialis*) that were exposed to different pollution sources (containing different proportions of dissolved PAHs and particulate PAHs) showed that accumulation patterns varied in relation to the source. The authors concluded that mussels located in waters in which mainly low MW soluble PAHs were present showed absorption mainly through the gills, whereas mussels located near the sediments in waters which were rich in high MW insoluble PAHs showed absorption patterns similar to those observed for food particles (Baumard et al., 1999). An early study was also undertaken on the distribution of PAHs in the clam *Rangia cuneata* following a 24-hour exposure period to C-14 labelled benzo(a)pyrene (BAP). The results demonstrated that the majority (75%) of the BAP was located in the viscera (comprising the digestive system, gonad and heart). The mantle, gills, adductor muscle and foot each contained between 3% and 16% of the BAP following the accumulation period (Neff and Anderson, 1975). The study did not note if the labelled BAP was associated with a food or particulate source when fed to the clams.

Dioxins and DL-PCBs appear to have similar tissue distribution patterns in bivalves to other contaminants. For example, to improve understanding of the biokinetics of dioxin uptake, distribution and elimination in the eastern oyster, experiments were undertaken using soluble radiolabelled 2,3,7,8-tetrachlorodibenzo-*p*–dioxin (TCDD), one of the most toxic dioxin congeners. The oysters were exposed to TCDD for 24 hours followed by a 28-day depuration period. The dioxin was observed to move rapidly via the oyster gills, into the haemolymph and then distributed through the other oyster tissues. The highest concentrations of dioxin throughout the experiment were detected in the digestive gland (Wintermyer et al., 2005).

In summary, shellfish take up contaminants (including metals, PAHs and dioxins/DL-PCBs) through two main pathways: direct absorption of soluble substances via the gills, and through ingestion of particulate bound contaminants. Thus the gills and digestive tissues generally harbour higher concentrations of chemical contaminants than other tissues immediately following exposure.

### 3.4.3 Comparison of contaminant accumulation between bivalve species

In order to compare the accumulation of the regulated contaminants by different species of bivalve shellfish, a literature search was undertaken using the search terms described in the Methodology (Section 2) and the search engines Google Scholar and Pubmed. There are many studies described in the literature that have investigated accumulation of contaminants in a single bivalve species, however because these results arise from independent studies undertaken under different environmental/ambient conditions, it can be difficult to evaluate the relative accumulation of different species. Therefore, in general, two types of studies have been reviewed; (1) those comparing the efficiency of accumulation of contaminants between bivalve species in the lab setting; and (2) field studies in which maximum concentrations of contaminants are provided for several species.

When comparing the efficiency of accumulation of contaminants in different bivalve species, it is necessary to consider the two main modes of accumulation, absorption through the gills and assimilation from ingested food sources, and the efficiencies associated with both mechanisms of uptake. Several terms are commonly used in the literature to describe the efficiency of these two processes: (a) assimilation efficiency (AE) refers to the uptake and accumulation of contaminants from ingested food sources, and absorption efficiency (α) refers to uptake of contaminants from the dissolved phase. Bioaccumulation of metals by bivalves has been shown to be directly proportion to the AE and is noted to be “a critical parameter to determine (and to make predictions of) bioaccumulation of chemicals from dietary exposure” (Wang and Fisher, 1999). There have been several studies that have investigated the AE and α of metals in different species of bivalves, these provide insight into the comparative ability of different species to accumulate contaminants and are discussed in the following sections.

Field studies in which concurrent monitoring of multiple bivalve species (i.e. different bivalve species collected at the same time and location) for regulated contaminants have been undertaken are also discussed – although, studies of this type are notably scarce in the literature. Some caution needs to be taken when comparing contaminant maxima between species and considering the appropriateness of a particular bivalve species (e.g. mussels) as an indicator, as bias may be introduced when certain species are sampled with a much higher frequency than others.

Studies that contribute significantly to the conclusions of this review have been subjected to a critical appraisal process to assist the reader to evaluate the usefulness of the findings of key publications. The process used for appraisal is detailed in the methodology (Section 3) and the findings of the critical appraisal are included in Appendix 1.

#### *Metals*

The AE of Cd and several additional metals were investigated through feeding radiolabelled plankton cells (*I. galbana* and *T. pseudonana)* to oysters (*C. virginica*), mussels (*M. edulis L.)* and two clam species (*Macoma balthica* and *M. mercenaria*). The exposure period was

between 40 and 60 minutes, following this the shellfish were depurated for two weeks. The AE for Cd in mussels (*M. edulis),* oysters (*C. gigas*) and clams (*M. balthica* and *M. mercenaria*) were: 11 – 40%, 69%, and 66 - 88% respectively (Reinfelder et al., 1997). The AE for Cd in clams and oysters exceeded that of mussels; *M. edulis* was found to retain less $^{109}$Cd than the other species of shellfish. The authors noted "this bivalve (*M. edulis*), the most widely employed organism in global bio-monitoring, is relatively inefficient at accumulating important elements such as Ag, Cd, and Zn from ingested phytoplankton." (Reinfelder et al., 1997). The AE for Hg and MeHg has also been reported for oysters, and was 2% and 55% respectively (reviewed in Wang and Fisher (1999)). This suggests that the AE for Hg in oysters may be lower than that of Cd in oysters (i.e. Cd is more efficiently accumulated by oysters than Hg).

Chong and Wang (2000) undertook a study in which they investigated the AE of Cd in two bivalves, the mussel *P. viridis* and the clam *R. philippinarum*. The bivalves were fed diets of five different diatom and dinoflagellate species and natural seston that had been radiolabelled with Cd. The Cd labelled algae were fed to the bivalves for 30 minutes followed by a three day depuration period. It was noted that for clams the AE varied by 2.5 fold according to the food type presented, similarly the AE of mussels also varied according to feed type by a factor of 2.3. The AE of Cd in clams was 1.8 – 4.7 times higher than that noted for the mussels; the AE of Cd in mussels was 11 – 25% whereas the AE of clams was 22 – 55%. The authors commented that the AE of Cd in the green mussel *P. viridis* (11 – 25%) was directly comparable to the AE of Cd in the common mussel *M. edulis* as established in other studies (11 – 40%). Similarly, the AE of the clam *R. philippinarum* was suggested to be consistent with other clam species and higher than that of *M. edulis.* It was also noted that the gut passage time of Cd was generally longer in clams than in mussels, which may relate to the higher AE in clams than mussels (Chong and Wang, 2000). These results are concurrent with the previous mentioned study in which the AE of clams and oysters exceeded that of mussels (Reinfelder et al., 1997).

Wang (2001) investigated the absorption efficiency (α) of a range of metals including Cd from the dissolved phase in two mussel species (*P. viridis* and *Septifer virgatus*) and the clam *R. philippinarum*. The α was less than 0.5 % for Cd for each of the three bivalves in the study. The α was found to vary widely within a species (i.e. large intra-specific variation), but inter-specific variation was not noted and it seems likely that the large intra-specific variation masked any between species differences in α. While interspecies differences in α were not apparent, other studies have shown significant inter-specific differences in α e.g. α was three to eight fold lower in clams than mussels (reviewed in Wang (2001)).

The literature demonstrates that the AE of metals from ingested sources exceeds that of the α from the dissolved phase. For example, for the clam *R. philippinarum,* the AE for Cd was reported to be between 22 and 55% (Chong and Wang, 2000), whereas the α was <0.5% (Wang, 2001). Similarly, the mean α of Cd directly from water by the mussel *M. edulis* was found to be 0.31%, whereas the AE from the consumption of food (radiolabelled natural seston) was found to be 8 – 20% (Wang et al., 1996). While the absorption of metals from ingested sources is more efficient than from soluble sources, accumulation from soluble sources may still contribute significantly to the overall metal loading due to the significant volumes of seawater that are pumped by bivalves.

Topping (1972) sampled a variety of areas in Scotland for a range of shellfish species including mussels (*M. edulis)* and scallops (*P. maximus* and *Chlamys* sp.), and analysed them for Cd and Pb. The highest level of Cd detected in scallops was 23 mg/kg, whereas the

highest level of Cd in mussels was 2 mg/kg. Most of the Cd was localised in the viscera of the scallop, with lower concentrations detected in the muscle and gonad. For Pb, the maximum concentration detected in mussels was 5.5 mg/kg, whereas the highest level recorded in scallops was 1.0 mg/kg (Topping, 1972). The author does not note if any of the mussel and scallop samples were collected concurrently (i.e. from the same location at the same time), so limited conclusions regarding the comparability of levels in mussels and scallops can be drawn. However, consistent with the results regarding high concentrations of Cd in scallops, Wang and Rainbow (2008) reviewed the AEs of a range of bivalves including oysters, mussels and scallops for Cd. It was noted that scallops contained the highest Cd concentrations, with mussels having the lowest and oysters having an intermediate concentration. AE values of mussels (*P. viridis*), oysters (*C. gigas*), scallops (*C. nobilis*) and King scallops (*P. maximus*) were noted to be 10 – 30%, 35 – 50%, 50%, and >80% respectively (reviewed in Wang and Rainbow (2008).

In 1996 concentrations of eight metals, including Cd and Pb, were investigated in three different bivalve species (the mussel *P. viridis*, the oyster *Crassostrea rivulans*, and the clam *R. philipinarum*) from 25 sites along the Pearl River Delta in the South China Sea. Not all species were collected from each site due to differences in the habitats, however collection of multiple species was possible at five of the sites. The concentrations of Cd in oysters were higher than those found in mussels or clams (maximum noted in oysters, mussels and clams = 50.91, 3.38 and 7.60 mg/kg respectively). Pb levels in oysters were noted to be low (maximum 0.98 mg/kg), whereas mussels and clams contained higher levels with maxima of 4.97 and 14.99 mg/kg respectively. The authors suggested that "oysters are different from mussels and clams with respect to their net uptake of Cd" and that oysters are mainly found in the estuarine regions, whereas mussels and clams are present in the inter-tidal zone and that these differences in habitat and feeding strategy may account for the differences in Cd uptake (Fang et al., 2003).

In-tank experiments were undertaken to investigate concentrations of Pb in two species of oyster (*C. gigas* and *Crassostrea margaritacea)* and two species of mussels (*Perna perna* and *Choromytilus meridionalis*) over a three-week period of exposure to soluble Pb (Watling, 1983). Results demonstrated that higher concentrations of Pb were accumulated by both mussel species (2.93 and 3.32 mg/kg) when compared to the oyster species (1.34 and 1.78 mg/kg). Accumulation of Cd was also investigated in the same study and the results were consistent with other studies, with oysters accumulating higher concentrations than co-exposed mussels (Watling, 1983).

Bioaccumulation of several trace metals, including Cd and Pb, by *M. galloprovincialis* and *O. edulis* growing at the same site in the Lim Fjord, Yugoslavia were investigated. Individual oysters and mussels which were around 1.5 years old were placed in cages and nets at a depth of 1.5 m and 25 animals of each species were sampled and tested for Cd and Pb. The length of time that the animals were placed in the environment prior to sampling was not stipulated. The results showed that the soft/edible tissue of oysters accumulated three fold higher Cd and two fold higher Pb concentrations than mussels (Martincié et al., 1984). The finding of higher concentrations of Pb in oysters compared with mussels is contrary to other findings noted above, which suggest that mussels may be better accumulators of Pb than oysters.

Oysters (*C. virginica* and *Crassostrea rhizophorae)* and mussels (*P. viridis)* were concurrently collected from six sites in the Gulf of Paria (Trinidad and Venezuela) and were analysed for various metals including Cd and Hg. Concentrations of Cd in oysters ranged between 0.02 mg/kg and 0.67 mg/kg and for mussels between <0.01 and 0.51 mg/kg. Notably, at

each of the six sites, co-sampled oysters were found to have higher levels of Cd than mussels, including three sites at which levels were reported to be <0.01 in mussels, but oysters contained between 0.02 mg/kg and 0.29 mg/kg. Concentrations of Hg in oysters were between 0.01 mg/kg and 0.07 mg/kg, and in mussels between 0.04 mg/kg and 0.11 mg/kg. Mercury concentrations at all six sites were found to be higher in mussels than oysters (Table 2 and 3 in Rojas de Astudillo et al. (2005)).

In summary, laboratory studies investigating the AE of metals through the consumption of plankton and other particulate matter consistently find that species of clams, scallops and oysters accumulate higher levels of Cd than mussel species through filter feeding processes. The α of Cd (i.e. absorption across the gill) is found to be higher in mussels than clams, however α is considerably lower than AE, which suggests that the uptake of metals from the soluble phase across the gill is not as significant as uptake via filter feeding and ingestion processes (e.g. AE). Field studies in which concurrent monitoring of metals in multiple bivalve species are scarce, however, three studies are reported in which co-sampling of oysters, mussels and clams was undertaken (Fang et al., 2003; Martincié et al., 1984; Rojas de Astudillo et al., 2005). These studies confirm the aforementioned laboratory results: oysters accumulated higher concentrations of Cd than mussels and clams.

Several field studies also demonstrated higher concentrations of Pb and Hg in mussels compared to co-sampled oysters (Fang et al., 2003; Rojas de Astudillo et al., 2005), Similarly, a laboratory study investigating comparative uptake of Pb by mussels and oysters also suggests higher rates of uptake of Pb by mussels compared with oysters (Watling, 1983); however, it is noted that one field study has suggested the converse regarding Pb – that is that native oysters accumulate higher concentrations than mussels (Martincié et al., 1984).

#### *PAHs, Dioxins and DL-PCBs*

Polycyclic aromatic hydrocarbons and PCBs were analysed in two different bivalve shellfish, *Macomona liliana*, a deposit feeder commonly called the wedge shell, and *Austrovenus stutchburyi*, a filter-feeding cockle. Five separate sites within the Manukau Harbour, New Zealand were concurrently sampled for both species. The results demonstrated similar concentrations of PAHs and PCBs in each species, however *M. liliana* generally contained slightly higher levels of both contaminants. The bioaccumulation factors for PCBs and PAHs were noted to be similar for the two species (Hickey et al., 1995).

Polycyclic aromatic hydrocarbons were monitored in three bivalve species (*Meretrix meritrix*, *Macoma birmanica* and *Sanguinolaria acuminata*) in the Sunderban mangrove wetland in India between January and February 2006 (Zuloaga et al., 2009). Three different sites were monitored, with one species collected from each site. Total PAH levels were found to be "significantly higher" in *S. acuminata* than the other two species. Bioaccumulation factors (the PAH level in bivalves divided by the PAH level in the sediments) was also highest in *S. acuminata* visceral tissue (48.60). While this study indicates a higher degree of bioaccumulation in *S. acuminata* than *M. meritrix* and *M. birmanica*, concurrent monitoring of all three species at the same site was not undertaken. Thus, in addition to differences in environmental PAH concentrations, the authors also note that differences in PAH concentration between species "could be caused by different physiological conditions and feeding habits in the bivalve population"(Zuloaga et al., 2009).

In 1996, the tanker the *Sea Empress* grounded near Milford Haven, south west Wales. Approximately 70,000 tonnes of oil contaminated over 200 km of coastline. A monitoring programme was put in place to evaluate contamination of seafood products in the area,

which included cockle beds, mussels and oysters. The shellfish were analysed for 19 different PAHs. The highest level reported was 6,800 mg/kg total hydrocarbons in mussels. Data on concurrently sampled species were not presented, however the authors note: “in general, where mussels and other molluscs (cockles and oysters) were sampled from the same site on a single occasion, the mussels contained the highest concentrations of total hydrocarbons and PAH” (Law et al., 1999).

A study was undertaken to investigate associations between the concentration of PAHs and PCBs and antioxidant enzymes in mussels (*M. galloprovincialis*), oysters (*Crassostrea* sp.) and crabs and mullets (Orbea et al., 2002). The study involved collection of samples from four separate locations in the Bay of Biscay during the summer and winter months of 1996/1997. Of the four locations sampled, oysters and mussels were concurrently collected from two of the sites. For the sites in which concurrent collection was undertaken, total PAH levels (both the sum of parental PAHs and sum of the alkylated PAHs) were found to be higher in oysters than mussels (with the exception of one site in winter). Similar results were found for PCBs, with oysters having higher levels than mussels. The authors noted “among the species examined in this work bivalves display the most appropriate features for monitoring purposes, and especially oysters, which accumulate organic contaminants to a greater extent and show more marked responses to environmental changes than mussels” (Orbea et al., 2002).

The bioaccumulation of PAHs and PCBs was investigated in the cockle (*Cerastoderma glaucum*), oyster (*Ostrea edulis*) and noble pen shell (*Pinna nobilis*) in nine areas within the Mar Menor lagoon in Spain (Leon et al., 2013). The aim of the study was to identify the most appropriate indicator species for organic pollutants in this area. Within the lagoon, nine sites were sampled, of which four were close to ports and urban areas, one was adjacent to a watercourse mouth and three were more distant from potential sources of pollution. Sampling of the shellfish was undertaken in the spring (June) and autumn (November) of 2010. Whenever possible sampling of all three species was undertaken, however due to shellfish stock availability, only three of the nine sites were concurrently sampled for all three species. The results showed that PAH content of the three species were generally very similar, with the exception of the spring sampling, when PAH levels in oysters were significantly higher than cockles. With regards to PCBs, higher concentrations were detected in the oyster and noble pen shell than cockles. The authors concluded that oysters represented the most appropriate indicator of organic pollutants in this area (Leon et al., 2013).

In summary, several studies have been undertaken in which concurrent sampling of multiple shellfish species was undertaken; these demonstrate that there is variation in the accumulation of organic contaminants between different bivalve species when they co-inhabit the same microenvironment. One study undertaken following an oil spill in the UK suggests that mussels ‘generally’ accumulate higher levels of PAHs than oysters and cockles (Law et al., 1999), however no data was presented to support this statement. A more recent investigation into comparative accumulation of PAHs and PCBs in oysters and mussels found that higher levels of accumulation generally occur in oysters when compared to mussels located at the same site, and led the authors to conclude that oysters were the most useful species for monitoring purposes (Orbea et al., 2002). Similarly, higher concentrations of PAHs and PCBs were found in oysters when compared to cockles from the same area (Leon et al., 2013).

### *Elimination of contaminants from bivalves*

Studies that have investigated concurrent elimination of contaminants in different bivalve species maintained under the same environmental conditions are scarce. Therefore, the following provides a brief overview of a selection of studies investigating contaminant elimination in individual bivalve species.

Elimination of Pb, Cd and Hg was investigated in black lip oysters (*Saccostrea echinata)* that were exposed to the metals over a 30-day period in tanks, and then subjected to a 30-day depuration phase. Depuration was undertaken at two different temperatures (20 and 30°C) and salinities (36 and 20 psu). The biological half-life ($b^1/_2$) is the time (days) it takes for half of the substance to disappear from the mollusc, thus gives a measure of elimination efficiency. The $b^1/_2$ values for Pb were between 13 and 49 days, for Hg between 24 and 136 days and for Cd between 30 and 203 days. This demonstrated that Pb was lost most rapidly from the oysters, followed by Hg and then Cd. The results also demonstrated that the $b^1/_2$ values obtained for different bivalve tissues (e.g. gill, digestive gland etc.) vary significantly (Denton and Burdon-Jones, 1981). The finding that Cd was retained for an extended time frame is consistent with the results of other researchers who also showed that oysters efficiently retain Cd.

Neff and Anderson (1975) undertook experiments in which they exposed clams to $C^{14}$ labelled PAH for 24 hours, and then subjected them to a depuration period of 58 days in clean seawater. Following 24 hours accumulation, clams had a level of 5.7 ppm BAP per animal; after six and 30 days depuration levels had dropped to 0.34 and 0.07 ppm respectively. Following 58 days depuration, no BAP could be detected in any of the five tissue types tested (viscera, mantle, gill, adductor and foot).

Wintermyer et al. (2005) utilised radiolabelled 2,3,7,8-tetrachlorodibenzo-*p*–dioxin (TCDD), one of the most toxic dioxin congeners, to investigate the biokinetics of uptake, distribution and elimination in the eastern oyster, *C. virginica*. The oysters were exposed to TCDD for 24 hours followed by a 28-day depuration period. The estimated $b^1/_2$ was between 14 and 24 days (Wintermyer et al., 2005).

Recently Gueguen et al. (2011) compiled $b^1/_2$ data for various contaminants in bivalve molluscs from various publications. The data compiled by Gueguen et al. (2011) demonstrates large variability in the $b^1/_2$ of contaminants between the various species studied, for example:

- *C. gigas* had a $b^1/_2$ of 137 days for Cd, whereas *Crassostrea belcheri* and *Crassostrea iredaleii* had $b^1/_2$ values of 5 – 16 days, and four days respectively for Cd.
- *M. galloprovincialis* had a $b^1/_2$ of 1000 days for Hg, whereas *C. gigas* had a $b^1/_2$ of 44 days.
- *M. edulis* had a $b^1/_2$ of 15 days for benzo(a)pyrene, whereas *C. virginica* had a $b^1/_2$ of 9-10 days.

It is stressed, the $b^1/_2$ values for individual species noted above were generated in separate studies, in which the animals were maintained in different conditions, and therefore direct comparisons may be confounded. Nonetheless, it seems probable from the data reviewed that large differences in elimination timeframes exist between species. Similarly, it is evident that there are significant differences in the elimination timeframes of different contaminants by the same bivalve species, and differences in elimination timeframes between different tissue types. Potential reasons for such differences are discussed in Section 3.4.4.

### 3.4.4 Possible reasons for interspecies differences in accumulation

Contaminant accumulation in bivalves is related to a range of factors that control uptake and sequestration in the tissues (e.g. bivalve feeding physiology and biology, environmental parameters and metal geochemistry) and to parameters that govern detoxification processes and toxin elimination. Wang (2001) concisely summarised the factors that affect metal accumulation in bivalves (Table 3.8). The following section provides a summary of the major parameters that have been documented to influence contaminant accumulation and elimination.

**Table 3.8:** Factors that potentially impact metal accumulation in bivalves from ingested food sources (assimilation efficiency) and from the soluble phase (absorption efficiency). Table reproduced from Wang (2001).

| Factors | Assimilation efficiency (from food) | Absorption efficiency (from water) |
|---|---|---|
| Bivalve feeding physiology and biology | Ingestion rate | Clearance rate |
| | Gut passage time | Gill surface area |
| | Digestive partitioning | Gill permeability |
| | Body size (small effect) | Body size (small effect) |
| Environmental parameters | Food quality | Salinity |
| | Food quantity | Osmolality |
| | Season | Dissolved organic carbon |
| Metal geochemistry | Phase speciation | Speciation |
| | Concentration | Concentration |
| | Metal-metal interaction | Metal-metal interaction |

#### *Variation in assimilation efficiency (AE)*

Various researchers have demonstrated that the AE for Cd is higher in oysters and clams than in mussels (Chong and Wang, 2000; Reinfelder et al., 1997; Wang and Fisher, 1999). Consistent with this finding, field studies have also demonstrated that concentrations of Cd in oysters are higher than that found in mussels grown in the same location (Fang et al., 2003; Rojas de Astudillo et al., 2005). Additionally, the AE for Cd in oysters was found to be higher than the AE for Hg in oysters, which is accordant with the results from depuration studies which demonstrate significantly longer biological half-life values for Cd in oysters compared with Hg (Denton and Burdon-Jones, 1981). Thus the AE appears to be strongly associated with final concentrations of contaminants in bivalves, and variations in the AE likely play a significant role in differences in the amount of contaminants accumulated by different bivalve species. In agreement with this hypothesis, food quality has been found to impact the AE, which has led to differences in the concentration of metals within bivalves, for example, Cd assimilation in *M. edulis* varied by three fold when the mussels were presented with different plankton diets (reviewed in Chong and Wang (2000)). Thus bivalves are likely to have varying AEs when presented with particular plankton diets/mixtures, which is likely to result in interspecies differences in contaminant accumulation.

#### *Interspecies differences in clearance and filtration rates*

Section 3.1.2 provides an overview of interspecies variation in the clearance rate (CR) and filtration rate (FR) of bivalve molluscs. Wang (2001) investigated the uptake rate and absorption efficiency (α) of a range of metals including Cd from the dissolved phase in two mussel species (*P. viridis* and *S. virgatus*) and the clam *R. philippinarum*. Mussels and clams were exposed to radiolabelled $^{109}$Cd for one and two hours, respectively, and measurements of Cd concentrations in water and individual bivalves were taken. The uptake rate (of Cd into the bivalve mantle cavity) was noted to be similar for the two mussel species, but 1.8 – 3.3 fold lower for clams. The authors also collated available published data on the metal uptake rate (into the mantle cavity) and CR for eight different bivalve species and showed a strong

linear relationship between the two variables. This demonstrated that the inter-specific variability in uptake of metals into the mantle cavity (prior to ingestion and absorption) was strongly linked to the differences in CR between species.

The absorption efficiency (α) was also strongly linked to the CR; a significant log-log negative relationship was found between α and the CR (Wang, 2001). This means that an increase in the bivalves filtration activity is coupled to a reduction in the efficiency in which metals are absorbed into the tissues. Thus while bivalves take up more metals from the dissolved phase into the mantle cavity (increased uptake rate), as the CR increases, the efficiency with which the metals are accumulated into the tissues declines (decreased α). All the metals included in the study of Wang (2001) (selenium, zinc and chromium and Cd) were noted to be similarly affected by the CR in each of the three bivalve species studied. The authors noted that the assimilation efficiency (AE) relating to metal uptake into bivalve tissues from consumed algae was also inversely related to CR.

Temperature and salinity also affects the CR and FR of bivalves, with different bivalve species having different optimal temperature and salinity ranges. It is likely that the interspecies differences in CR and FR, which are exacerbated by temperature and salinity, result in variations in the absorption of chemical contaminants between species. Consistent with this idea, variations in temperature and salinity have been documented to impact the absorption of metals by bivalves. The black lip oyster, *S. echinata*, was exposed to 10 µg/L Hg, Cd and Pb (in solution) at 30°C and 20°C, at two different salinities (36 psu and 20 psu). Exposure was for 30 days followed by a depuration period of 30 days (Denton and Burdon-Jones, 1981). At both salinity levels, accumulation of Hg and Cd was significantly greater at the higher temperature than the lower temperature (in all tissues tested), Pb marginally increased at the higher temperature. Metal accumulation was generally higher at the lower salinity level of 20 psu, than 36 psu (Denton and Burdon-Jones, 1981). Several more recent studies regarding the impact of salinity on metal uptake by bivalves were reviewed by Wang and Rainbow (2008), which also suggest that mussels (*P. viridis*) from lower salinity sites have higher Cd tissue concentrations that mussels obtained from higher salinity sites. Salinity may also affect the available state of the metal (speciation) and thus impact metal uptake, as well as the bivalve physiology. Thus salinity and temperature both appear to be associated with the concentration of metals assimilated in bivalves. It seems most likely that the impact of temperature and salinity on metal uptake in bivalves is related to the changes in CR and FR, and will vary markedly between bivalve species.

### *Seasonal variations in uptake of contaminants*

Following the 1996 grounding of the tanker *Sea Empress* in Wales, a monitoring programme for PAHs in shellfish was established. As part of this programme, benzo(a)pyrene (BAP) was analysed in mussels over a period of 500 days at two separate sites in Wales. Seasonal patterns in BAP levels were observed at both sites, with higher concentrations observed over winter. It was noted that the seasonality pattern was similar to that reported for mussels from Germany (Law et al., 1999). Three factors were suggested to contribute to the seasonal variability:

- Increased concentrations of bioavailable PAH in the water column due to increased environmental inputs from power generation, greater run-off and freshwater inputs etc.;
- The decrease in mussel metabolic capacity in winter leads to decreased enzymatic activity and less detoxification; and
- Increased lipid storage in winter in preparation for spawning in spring (Law et al., 1999).

Similar findings were reported by Webster et al. (2006) from a long term monitoring project based in a remote Scottish site (Loch Etive). Samples of *M. edulis* were collected on a monthly basis between 1999 and 2005 and analysed for PAHs. Concentrations of PAHs were significantly higher in samples collected during the winter months between November and March, than those collected between April and October. The authors related this finding to the reproduction cycle of mussels, suggesting that the increase in lipids for the purpose of gametogenesis results in an increase in the uptake of hydrophobic (lipophilic) contaminants, and that spawning in late spring/early summer results in a decrease in PAH load (Webster et al., 2006).

Consistent with these findings, Capuzzo et al. (1989) monitored concentrations of PCBs in mussels (*M. edulis*) from two sites in the USA over a one year period. Levels were found to fluctuate during the year with a notable decrease in the autumn months, which was correlated with the seasonal reproductive cycle of the mussel (spawning) and associated variations in lipid content (Capuzzo et al., 1989). Similar to mussels, a relationship between PCB accumulation in oysters (*C. virginica*) was correlated with lipid content (Chu et al., 2003). Orbea et al. (2002) also describe elevated concentrations of PCBs and PAHs in oysters (*Crassostrea* sp.) and mussels (*M. galloprovincialis*) during winter compared to summer. Similar to mussels, elevated PAH levels in oysters during winter have also been associated with gametogenesis: the eggs and sperm of *C. virginica* were shown to contain high concentrations of PAHs compared to somatic tissues and were found to lose up to 50% of the PAH present following spawning (Ellis et al., 1993).

The reverse pattern is suggested to occur for inorganic contaminants such as the metals; that is when the bivalves reach sexual maturity and the bivalves body mass has increased, there is a dilution effect because the level of metal remains the same (thus total concentrations decrease). This has been reported for both Cd and Pb in mussels and oysters (reviewed in Gueguen et al. (2011)).

Given the noted seasonal occurrence of contaminants in bivalves, which relates to sexual maturity, significant changes in the concentration of a particular contaminant can occur directly following bivalve spawning. Variation in the times at which bivalves sexually mature may result in interspecies differences in accumulation of contaminants by bivalves, though no direct research appears to have been undertaken to specifically test this hypothesis.

### *Exposure to contaminants*

As noted previously, shellfish that are commercially produced in Scotland occupy different marine habitats (e.g. some inhabit the seafloor, others are grown subtidally on long lines, and others are intertidal) (Table 3.4). These differences in habitat are likely to result in differences in exposure to certain contaminants. For example, species that live within the sediments, such as surf clams and cockles, may experience heightened exposure to sediment bound contaminants compared to bivalves that live within the water column such as rope grown mussels, which may be more exposed to soluble contaminants.

A study undertaken by Næs et al. (1998) supports this idea. Principal component analysis was used to investigate patterns of PAH accumulation in four bivalve species for which data had been gathered over a 20 year period from seven fjords and coastal areas impacted by metal smelters in Norway. The dataset included some 272 samples of *M. edulis* (common mussel), *Modiolus modiolus* (horse mussel), *Littorina littorea* (periwinkle) and *Patella vulgata* (limpet). A suite of 12 PAHs that were consistently analysed in each species were used for the data analysis. Of the species analysed, two or three species were concurrently

sampled in some fjords, and the concurrent data was used to examine differences in the profile of compounds present in each species. The common mussel, limpet and periwinkles were noted to have similar profiles, however horse mussels were shown to contain high proportions of the heavier PAH compounds. Compounds of higher molecular weight are less soluble, and more likely associated with particulate matters within the sediments. The authors suggested that the difference in profile between species may be related to the depth and habitat preferences of the bivalves sampled, with common mussels, limpets and periwinkles commonly found on rocky shore habitat, but horse mussels found sub-tidally and partially buried in substrate (Næs et al., 1998). Thus, horse mussels may be more exposed to the higher molecular weight compounds. Similarly, a study undertaken by Baumard et al. (1999) found that mussels grown in different locations which were exposed to different contamination sources (particulate bound contaminants versus soluble contaminants), showed different patterns of accumulation.

Associations between habitat and contaminant concentrations in different bivalve species does not appear to have been extensively researched in the literature, however some researchers note that this may account for interspecies differences. For example, Fang et al. (2003) note that Cd levels are much higher in oysters than mussels and clams collected through the Pearl River Delta in South China. The authors suggested that this difference is related to the habitat of the species, with oysters growing largely in estuarine areas whereas mussels and clams grow in the intertidal zone.

### *Detoxification and elimination*

Bivalves that are exposed to metals generally attempt to limit/reduce toxic effects (to themselves) through two mechanisms. The first involves transforming the metal into a salt so that it is immobilised and thereby limits its distribution in the body. The second mechanism is the induction of metallothioneins (MT). Both Cd and Hg can induce MTs, but their induction is variable both within and between bivalve species. The MT forms a complex with the metal, which renders it harmless to the bivalve. These mechanisms enable bivalves to exist in highly contaminated environments and accumulate high concentrations of metals (reviewed in Gueguen et al. (2011) and Amiard et al. (2006)). The mechanisms employed to detoxify metals however, do not protect the bivalve consumer from the toxic effects of the metal, which will often be released from the complexed form upon consumption of the bivalve. Some invertebrates are also noted to be able to detoxify organic pollutants such as PAHs, dioxins and PCBs primarily through oxidation and conjugation processes that take place in particular organs. Although, it is suggested that bivalves are not able to metabolise PAHs, which may account for its prolonged retention in bivalves (Fernandes et al., 2013).

If a metal is bound to MT, or stored within a bivalve in a detoxified form (as is the case for Cd in some species of scallops), the elimination rate may be lower, particularly if the metal has been transformed to an insoluble state (reviewed in Wang and Rainbow (2008)). In this recent review by Wang and Rainbow (2008), it was noted that 80% of Cd is stored in the digestive gland of *P. maximus* in the soluble form and it is eliminated with a $b^1/_2$ of four months, whereas 60% of the Cd accumulated by the scallop *C. varia* is stored in the insoluble form and is effectively retained indefinitely. Thus the detoxification processes used by bivalves vary between species, and may play a role in interspecies differences in total contaminant concentrations (reviewed in Gueguen et al. (2011)).

Similar to the kinetics of contaminant accumulation, the kinetics of contaminant elimination (purging of contaminants when in clean uncontaminated seawater) depend on a variety of different factors, including:

- The concentration of contaminant present;
- The mode of contamination (through water or food ingestion);
- The growth rate of the organism;
- Sexual maturity status;
- Water temperature; and
- Quantity and type of food available.

Given the range of factors involved in controlling contaminant elimination, a review by Gueguen et al. (2011) noted that 'it is obvious that the elimination kinetics, the mechanisms of elimination, and quantities of toxicants eliminated will be species-dependent." This statement is supported by data on the biological half-life of contaminants in shellfish, which shows large interspecies variations (Section 3.4.3).

### 3.4.5 Summary of potential issues associated with the use of indicators

The findings of the literature review are considered in the context of several of the major criterion proposed by Schwacke et al. (2013) for evaluating the effectiveness of marine indicator species (Section 3.2):

***Criterion 1 'Sensitivity for bioaccumulation': an indicator species should be highly sensitive to concentration of the contaminant, and be more sensitive than the other shellfish species of interest.***

The previous sections demonstrate that when concurrent monitoring was undertaken significant interspecies differences in contaminant accumulation are noted:

- For Cd, oysters accumulated higher concentrations than mussels in several field studies (Fang et al., 2003; Martincié et al. 1984; Rojas de Astudillo et al., 2005). Laboratory studies also suggest that assimilation of Cd is higher for clam and scallop species than mussels (Reinfelder et al., 1997; Wang and Rainbow et al., 2008; Watling 1983).
- For Hg and Pb, generally higher concentrations have been observed in mussels compared to co-sampled oysters (Fang et al., 2003; Rojas de Astudillo et al. 2005; Watling 1983); however, one study notes the converse for Pb i.e. oysters accumulated higher levels than mussels (Martincié et al., 1984).
- In relation to organic contaminants, one study involving concurrent monitoring of oysters and mussels found higher levels of accumulation of PCBs and PAHs in oysters compared to mussels and concluded that oysters were the most useful species for monitoring purposes (Orbea et al. 2002). Similarly, a study suggests higher levels of PCBs and PAHs in oysters as compared to cockles (Leon et al., 2013).

An evaluation of the robustness of the publications noted above has been undertaken (Appendix 1).

Collectively these results suggest that the choice of indicator species may be dependent on the particular chemical contaminant of interest, with some contaminants accumulating more efficiently in oysters, and others in mussels. With respect to Cd, the results of this review suggest that oysters are the most sensitive bivalve species and may be an appropriate indicator to use in locations where multiple species are grown.

While there is significant data pertaining to the preferential accumulation and retention of Cd in oysters compared to mussels, comparative data regarding accumulation of Hg, Pb and the organic contaminants is limited, with only a few studies in which concurrent monitoring of multiple species have been undertaken. It is also emphasised that few studies involving

concurrent monitoring of contaminants in multiple bivalve species have included the other commercial species of interest in Scotland e.g. scallops, surf clams and cockles. This represents a significant data gap in this analysis. Thus further studies regarding comparative accumulation between species would be needed to fully evaluate the most sensitive species for Pb, Hg and the organic contaminants.

With respect to contaminant elimination, the review highlights large apparent differences in elimination timeframes for contaminants by different shellfish species; however, these data were generated in separate studies of bivalves maintained in different environmental conditions. There is a lack of data regarding contaminant elimination by bivalves that are co-located and exposed to the same environmental conditions. The lack of such data prohibits analysis regarding the appropriate bivalve species to use as an indicator of contamination following an environmental contamination event such as an oil spill – the obvious choice being a species that exhibits prolonged biological half-life values. This finding is consistent with a recent review undertaken by Gueguen et al. (2011) which notes: "considerably more research results are needed to achieve reliable estimates of the half-lives in shellfish species of the main contaminants found in the marine environment."

***Criterion 2 'Appropriate distribution': Indicator species should have a distribution that overlaps with the other shellfish species of interest.***

Table 3.4 provides an overview of the preferred habitat of commercial shellfish stocks in Scotland. It can be seen from this summary that some species such as mussels are grown suspended in the water column from long lines, others are grown on the seafloor (e.g. scallops), and others inhabit the intertidal zone (e.g. oysters and clams). Notably cockles and surf clams live within the sediments and may experience higher exposure to particulate bound contaminants than bivalve species which are suspended in the water column. These differences in habitat may mean that different bivalve species have different patterns of exposure to particulate bound and soluble contaminants (Baumard et al., 1999), which could result in interspecies differences in contaminant concentrations. As noted previously, the data is very limited regarding associations between contaminant concentrations and habitat. Given the data limitations, it may be appropriate to undertake concurrent sampling of bivalves within the same area to ascertain if habitat differences result in significant differences in accumulation, this may be particularly important for areas in which sediment dwelling organisms are commercially harvested, such as scallop, surf clam and cockle sites.

# SECTION FOUR: INTERNATIONAL USE OF INDICATOR SPECIES IN RISK MANAGEMENT PROGRAMMES

To gain insight into the global use of indicator species in management programmes, a small survey was prepared and distributed to a number of shellfish producing countries (Appendix 2). Eleven of the 12 countries that returned responses use indicator species within their shellfish marine toxin risk management programme (Table 4.1). Northern Ireland does not use indicator species, it does have areas in which multiple species are produced, and these are presumably monitored separately. All other countries returning responses to the survey use indicator species within their toxin management programmes. A previous survey was undertaken in 2011-2012 on the use of indicator species for marine toxin management within European Union member countries. The 2011-2012 survey indicated that of the nine respondents that have shellfish production areas within their countries, five used indicator species and four did not. Italy, Denmark, Norway and Romania did not use indicator species. Denmark and Norway were noted to have multi-species production areas in which all species are tested separately (Kasia Kazimierczak, personal communication, August 2014).

Ten of the 12 countries that returned responses to the 2014 survey do not use indicator species within their chemical contaminant management programmes. Two countries (Netherlands and USA) noted that mussels were used as an indicator for metals, PAHs, dioxins and PCBs.

The following sections provide an overview of the major results obtained from the 2014 survey.

## 4.1 Species used as indicators for marine toxin management

All countries that utilise indicator species (both the current survey and 2011/12 survey) use mussels as an indicator for a wide variety of other shellfish species (including oysters, scallops, clams and cockles) and marine toxin types (Table 4.1). Two countries (Canada and Scotland) use mussels as an indicator species for nearby scallops, however neither countries have concurrent monitoring data for mussels and scallops to demonstrate relative accumulation and elimination rates. Ireland and New Zealand noted that they do not use indicator species for scallops, in part due to concerns over significant differences in toxin profiles between scallops and mussels (for Ireland, particularly in relation to ASP).

Interestingly, the 2014 survey highlights that five countries also use species other than mussels as indicator organisms in some areas (Table 4.1). Non-mussel species used as indicators included (species names not noted):

- Oysters (both Pacific and Native): used to represent toxins in cockles, oysters, clams and mussels;
- Cockles: used to represent toxins in mussels, clams, razors and native oysters; and
- Clams: used to represent toxins in native oysters, and other clam species.

Of the eleven countries that utilise indicator species (2014 survey), seven noted that concurrent monitoring data was available to support the use of the chosen indicator species, of these two countries have published the data in peer reviewed journals.

## 4.2 Approach to marine toxin management using indicator species

Respondents noted a variety of management actions taken in response to the detection of marine toxins in indicator species (Table 4.2). If toxin levels are detected in the indicator species at concentrations less than the maximum permissible level (MPL) the regulatory responses taken by various countries include:

- No action;
- Commencement of testing of other species growing in the same area;
- Testing other species in the same area at an increased frequency; and
- Testing the indicator species at an increased frequency.

If toxin levels are detected in the indicator species at concentrations greater than the MPL the regulatory responses taken by various countries include:

- Harvesting restrictions for all species in the area;
- Harvesting restricted for indicator species, testing of other species in the same area is initiated (but closure not applied);
- Harvesting restrictions for all species in the area, other species tested to assess if closure appropriate.

Eight of the 11 countries that use indicator species noted that following the closure of a production area due to a toxin event, each species is tested to show they are no longer toxic (Table 4.2). England, Wales and Scotland generally test the indicator species and use this result to open production areas for all species that are present. For England and Wales, in some cases additional samples of other classified species in an area may be monitored for re-opening, particularly if significant commercial harvesting of other species is undertaken.

Ten of the 12 survey respondents noted that phytoplankton was used as an indicator of shellfish toxicity within the marine toxin management programme. None of the respondents reported using other technologies, such as solid phase adsorption toxin tracking devices, to indicate potential toxicity within production areas. One respondent noted the use of molecular probes to indicate the presence of *A. spinosum* and toxic *Pseudo-nitzschia* spp.

## 4.3 Potential issues identified with the use of indicator species

The survey included the following question to assist in identification of potential issues associated with the use of indicator species:

*"Have there been any instances in which an indicator species has tested negative (or been below the regulatory limit) and 'other' species of shellfish present in the same area have exceeded the regulatory limit for a particular toxin type?"*

Table 4.2 provides a summary of responses to this question. Two respondents were unsure whether such a circumstance had occurred. Four respondents stated that there had been no instances of toxicity in other species in the absence of toxicity in the indicator species. Five respondents noted that there had been instances of toxicity in other species when the indicator species was either negative or of low toxicity, examples of this were provided by four of the respondents:

- High levels of DSP were observed in clams (*Plebidonax deltoides*) when nearby oysters used as an indicator species were negative (1 km separation between species was noted);

- Scallops were noted to depurate more slowly and remain toxic[10] for longer than mussels;
- It was noted that there have been a few occasions in which mussels were negative and other species were positive[10]; and
- Oysters were noted to retain toxin for longer than mussels, and mussels thus may be negative while oysters are still toxic[10].

[10]Toxin type(s) not specified.

**Table 4.1:** Use of indicator species in a selection of shellfish producing countries: shellfish species used as indicators, species represented by indicators, and availability of validation data to support the selection of a particular species as an indicator. NA = Not applicable.

| Country | Are indicator species used? | What species is used as an indicator? | What species are represented by the indicator? | Are scallops represented by the indicator? | Is concurrent monitoring data available to support the use of the indicator species? | Is the validation data published? |
|---|---|---|---|---|---|---|
| Australia | 1. Yes (New South Wales, South Australia and Tasmania)<br>2. No (Western Australia and Queensland) | 1. Oysters (NSW)<br>2. Mussels (Tasmania)<br>3. Pacific oysters (SA) | 1. Cockles (NSW)<br>2. Oysters, clams, scallops and lobsters (Tasmania)<br>3. Native oysters and cockles (SA) | Yes (Tasmania) | No (NSW)<br>Yes (SA)<br>Very limited data (Tasmania | No |
| Canada | Yes | Mussels | Clams, scallops, mussels | Yes | No | NA |
| England & Wales | Yes | 1. Mussels<br>2. Cockles<br>3. Pacific oysters<br>4. Native oysters<br>5. Clams | 1. Oysters, cockles, clams and razors<br>2. Mussels<br>3. Cockles, native oysters, hard clams, mussels<br>4. Pacific oysters, hard clams, mussels<br>5. Native oysters, hard clams, manila clams | No | No (noted that limited concurrent monitoring data is available but has not been analysed) | NA |
| France | Yes | Mussels (lipophilic toxins only) | All other shellfish species | Yes (coastal production)<br>No (offshore scallops) | Yes | Some data is published |
| Ireland | Yes | Mussels | Oysters, clams and cockles | No | Yes | No |
| Northern Ireland | No | NA | NA | NA | NA | NA |
| New Zealand | Yes | Mussels | Clams, oysters (Pacific and flat), mussels, geoducks | No | Yes | No |
| Portugal | Yes | 1. Mussels<br>2. Cockles | 1. Cockle, clams, razors, native oysters<br>2. Mussels, clams, razors and native oysters | No | Yes | Yes |
| Scotland | Yes | 1. Mussels<br>2. Cockles<br>3. Pacific oysters<br>4. Razors | 1. Pacific oysters, cockles, King scallops, native oysters, razors<br>2. Razors, pacific oysters<br>3. Mussels, Queen scallops, razors<br>4. Cockles | Yes | No | NA |
| Sweden | Yes | Mussels | Native oysters, Pacific oysters | No | Yes | Yes |
| The Netherlands | Yes | 1. Mussels<br>2. Oysters | 1. Oysters<br>2. Cockle | No | Yes | No (publication in prep.) |
| The USA | Yes (some States do) | Not specified | Not specified | No | Not specified | Not specified |

**Table 4.2:** Risk management responses following the detection of marine toxins in shellfish indicator species

| Country | Follow up actions if toxin is present in indicator at concentrations < MPL | Follow up actions if toxin is present in indicator at concentrations > MPL | Are indicator species used to re-open areas? | Have there been instances in which the indicator is below the MPL, but other species have been above MPL? |
|---|---|---|---|---|
| Australia | Generally no action if < MPL<br>New South Wales and South Australia: Would test other species in area | Harvesting is restricted for all species<br>Further testing of other species may follow to allow closures for certain species only | No | Yes - New South Wales: high levels of DSP observed in clams (*Plebidonax deltoides*) when oysters negative (1 km separation)<br>No - Tasmania |
| Canada | No action if < MPL | Harvesting is restricted for all species | No | Yes – in cold water, scallops depurate slower and were toxic longer than mussels |
| England and Wales | If level is > ½ MPL weekly testing of indicator species is triggered. No action if < ½ MPL | Harvesting is restricted for all species | Yes. Indicator species tested for re-opening. Other species may also be monitored depending on local considerations | Yes – there have been a few occasions in which mussels have been negative but other species have been positive |
| France | No action if PSP and ASP <MPL<br>Lipophilic toxins: other shellfish species tested if level is > ½ MPL | Harvesting is restricted for all species | No | No |
| Ireland | Frequency of testing of other species in the area is increased | Harvesting of indicator species is prohibited, testing of other species is conducted (but closure not generally applied) | No. Each species is tested individually to show they are no longer toxic | Yes – oysters retain toxin for longer than mussels, and mussels may be negative while oysters are still toxic |
| Northern Ireland | NA | NA | NA | NA |
| New Zealand | May test other species in area | Area closed for all species pending sampling from other commercial species | No. Each species is tested individually to show they are no longer toxic | No |
| Portugal | If <MPL testing frequency is increased to weekly. If plankton detected, sampling increased for other shellfish species | Harvesting is restricted for all species | No – all species are tested to show they are no longer toxic | Yes |
| Scotland | If level is > ½ MPL weekly testing of indicator species is triggered. No action if < ½ MPL | Harvesting is restricted for all species | Yes. Indicator species is tested and the result used to open areas for all species | Unknown |
| Sweden | If level is > ½ MPL weekly testing of indicator species is triggered. | Harvesting is restricted for all species until other species are tested and show levels below ½ the MPL | No | No |
| The Netherlands | Sampling of other species initiated | Harvesting is restricted for all species. Sampling of other species will be undertaken | No. If toxins occur, each species will be tested individually to show they are no longer toxic | No |
| The USA | Frequency of testing usually increased | Not specified | Not specified | Unknown |

# SECTION FIVE: REVIEW OF HISTORICAL MONITORING DATA

A review and analysis of marine toxin data that has been collected through the Official Control programme (facilitated by the FSA) has been undertaken. The methodology used to review the data is detailed in Section 2 of this report. The following sections provide a summary of the key findings from the review of data.

## 5.1 Analysis of marine toxin data

### 5.1.1 Pods in which indicators are used

Table 5.1 provides an overview of the number of regions (referred to as pods) in Scotland in which indicator species are used for toxin management purposes. Of the 84 pods, 26 use an indicator species approach for management, of which seven are single species pods (as of March 2014). Indicator species are used in single species pods in which the commercially harvested species is difficult to access for routine sampling purposes.

**Table 5.1**: Summary of the number of marine toxin management areas ('pods') in Scotland that use indicator species. Information presented is based on the classified production areas and representative monitoring species as of March 2014.

| | |
|---|---|
| Total number of pods | 84 |
| Number of single species pods | 64 |
| Single species pods in which indicators are used | 7 |
| Number of multi species pods | 19 |
| **Total number of pods in which indicators used** | **26** |

The majority of pods in which an indicator species is used employ mussels as the sentinel species (19 pods). Mussels are used to represent Pacific oysters, cockles, King scallops, native oysters and razors in these pods (Table 5.2). Pacific oysters are used as an indicator species in three pods and represent mussels, Queen scallops and razors in these areas. Cockles are used as an indicator species in three pods, and represent razors and Pacific oysters. Razors are used as an indicator species for cockles in one pod. Table 5.2 provides a summary of the species used as indicators in Scotland. Table 5.3 also shows a summary of species used as indicators, but incorporates backup monitoring sites that are utilised when the usual monitoring site cannot be accessed or the usual species sampled is no longer available. The data analysis undertaken focuses on the various combinations of indicator and representative species noted in Table 5.2.

**Table 5.2:** Indicator species used in Scotland. Species represented by the indicator and the number of pods in which the indicator/representative species combination is used. Information presented is based on the classified production areas and the **usual** representative monitoring species used, as of March 2014.

| Indicator species | Representative species | Number of pods |
|---|---|---|
| Mussels | Pacific oysters | 12 |
| | Cockles | 7 |
| | King scallops | 2 |
| | Native oysters | 2 |
| | Razors | 2 |
| Cockles | Razors | 3 |
| | Pacific oysters | 1 |
| Pacific oysters | Mussels | 1 |
| | Queen scallops | 1 |
| | Razors | 1 |
| Razors | Cockles | 1 |

**Table 5.3:** Indicator species used in Scotland. Species represented by the indicator and the number of pods in which the indicator/representative species combination is used. Information presented is based on the classified production areas and the **usual** and **alternate** representative monitoring species used, as of March 2014. Alternate monitoring species may be used if a sampling officer cannot access the usually monitored species, if the usual species becomes too limited in numbers to collect, or for other reasons.

| Indicator species | Representative species | Number of pods |
|---|---|---|
| Mussels | Pacific oysters | 13 |
| | Cockles | 8 |
| | Razors | 3 |
| | King scallops | 2 |
| | Native oysters | 2 |
| | Surf clam | 1 |
| | Queen scallop | 1 |
| Pacific oysters | Mussels | 4 |
| | Razors | 2 |
| | Queen scallops | 1 |
| | Native oysters | 1 |
| Cockles | Razors | 3 |
| | Pacific oysters | 1 |
| Razors | Cockles | 2 |

### 5.1.2 Summary of marine toxin data: October 2010 – December 2013

An initial evaluation of all marine toxin data for commercially produced bivalve species generated from the routine official control programme was undertaken. The evaluation included collating the following information for each commercial species and regulated toxin group:

- The number of samples tested;

- The number of samples in which a toxin had been detected;
- The number of samples exceeding the MPL and ½ MPL; and
- The maximum concentration detected.

PSP and ASP data generated between October 2010 and December 2013, and lipophilic toxin (DSP, ASP and YTX) data generated between July 2011 and December 2013, were evaluated. The timeframe selected for the evaluation represents a period in which the analytical methods used for toxin testing were consistent;

- For PSP an LC-FLD method based on the AOAC official method 2005.06 was used;
- For ASP an LC-UV method was used; and
- For the lipophilic toxins (DSP, AZA and YTX) an LC-MS/MS method was used in accordance with conditions specified by the EU Reference Laboratory (Stubbs et al., 2014).

Tables 5.4 to 5.8 present summaries of the information collated for each commercial shellfish species produced in the in-shore environment. It should be noted that no samples of native oysters or King scallops (in-shore) were collected and tested for toxins as part of the official control programme over this period. Only 11 samples of Queen scallops were collected and tested and therefore, were omitted from the summaries due to low sample numbers. For PSP, DSP, AZA and YTX the highest concentrations were recorded in mussels; for ASP the highest concentration observed occurred in cockles.

The proportion of samples in which PSP was detected was between 4.2% and 11% for mussels, razors, Pacific oysters and cockles (Table 5.4).

**Table 5.4:** Summary of **PSP** detections in each species commercially harvested in Scotland (October 2010 – December 2013). Data generated from HPLC testing (not mouse bioassay). RL = Reporting Limit. MPL = Maximum Permissible Level.

| | **Mussels** | **Razors** | **Pacific oysters** | **Cockles** | **Surf clams** |
|---|---|---|---|---|---|
| | *Mytilus* spp. | *Ensis* sp. | *Crassostrea gigas* | *Cerastoderma edule* | *Spisula solida* |
| Number of samples analysed and reported | 4491 | 392 | 1032 | 364 | 73 |
| Number of samples PSP Not Detected[a] | 4120 (91.7%) | 352 (90%) | 989 (95.8%) | 324 (89%) | 53 (72.6%) |
| Number of samples PSP Detected[a] | 371 (8.2%) | 40 (10.2%) | 43 (4.2%) | 40 (11%) | 20 (27.4%) |
| Number of samples < RL[b] | 262 (5.8%) | 27 (6.9%) | 40 (3.9%) | 30 (8.2%) | 14 (19.2%) |
| Number of samples > RL[b] | 109 (2.4%) | 13 (3.3%) | 3 (0.3%) | 10 (2.7%) | 6 (8.2%) |
| Number of samples ≥ ½ MPL[c] | 64 (1.4%) | 5 (1.3%) | 1 (0.1%) | 3 (0.8%) | 5 (6.9%) |
| Number of samples ≥ MPL[c] | 43 (1%) | 1 (0.3%) | 0 (0%) | 2 (0.5%) | 3 (4.1%) |
| Maximum concentration detected (µg/kg) | 4776 | 1571 | 572 | 1678 | 2152 |

[a]Number of samples for which sample was 'not detected' and 'detected' using the HPLC qualitative screen test. Samples that produce a 'detected' result by the HPLC screen test are subjected to the HPLC quantitative test.
[b]Number of samples that produce results < and > the reporting limit (RL) of the HPLC quantitative test. Percentage values are derived using the total number of samples analysed as the denominator.
[c]The number of samples ≥ ½ MPL and ≥ MPL do not add to give the total number of samples >RL. There are additional samples that produced values between the RL and ½ MPL, and some samples ≥ ½ MPL are also ≥ MPL.

A higher proportion of surf clams contained PSPs, with 27.4% of samples having detectable concentrations of PSP using the HPLC screen method. Similarly, the proportion of samples with PSP concentrations above the MPL was between 0 and 1% for mussels, razors, Pacific oysters and cockles, but 4.1% of surf clams tested had concentrations higher than the MPL (Table 5.4).

DSP was infrequently detected in razors, Pacific oysters and cockles; between 1.3% and 4.1% of samples had levels exceeding the reporting limit (RL) (Table 5.5). Whereas 28.1% and 56.9% of mussels and surf clams respectively, had concentrations exceeding the RL for DSP. The proportion of mussel and surf clam samples containing levels above the MPL was 9% and 7.7% respectively (Table 5.5).

**Table 5.5:** Summary of **DSP** detections in each species commercially harvested in Scotland (July 2011 – December 2013). Data generated from LC-MS/MS testing only (not mouse bioassay). RL = Reporting Limit. MPL = Maximum Permissible Level.

| | **Mussels** | **Razors** | **Pacific oysters** | **Cockles** | **Surf clams** |
|---|---|---|---|---|---|
| | *Mytilus* spp. | *Ensis* sp. | *Crassostrea gigas* | *Cerastoderma edule* | *Spisula solida* |
| Number of samples analysed and reported | 5175 | 367 | 1173 | 335 | 65 |
| Number of samples < RL | 3721 (71.9%) | 352 (95.9%) | 1158 (98.7%) | 324 (96.7%) | 28 (43.1%) |
| Number of samples DSP >RL | 1454 (28.1%) | 15 (4.1%) | 15 (1.3%) | 11 (3.3%) | 37 (56.9%) |
| Number of samples ≥ ½ MPL[a] | 842 (16.3%) | 8 (2.2%) | 8 (0.7%) | 2 (0.6%) | 19 (29.2%) |
| Number of samples ≥ MPL[a] | 467 (9%) | 2 (0.5%) | 3 (0.3%) | 0 (0%) | 5 (7.7%) |
| Maximum concentration detected (µg/kg) | 6950 | 231 | 682 | 146 | 256 |

[a]The number of samples ≥ ½ MPL and ≥ MPL do not add to give the total number of samples >RL. There are additional samples that produced values between the RL and ½ MPL, and some samples ≥ ½ MPL are also ≥ MPL.

AZA was infrequently detected in razors and cockles with no samples exceeding the MPL (Table 5.6). A relatively high proportion of Pacific oyster and surf clam samples had concentrations exceeding the RL of AZA, 24.4% and 50.8% respectively, compared with 4.8% of mussel samples. Similarly, the proportion of samples above ½ MPL was high for Pacific oysters and surf clams, 12.2% and 15.4% respectively, compared to 1.8% of mussel samples (Table 5.6).

**Table 5.6:** Summary of **AZA** detections in each species commercially harvested in Scotland (July 2011 – December 2013). Data generated from LC-MS/MS testing (not mouse bioassay) is included in the table. RL = Reporting Limit. MPL = Maximum Permissible Level.

| | **Mussels** *Mytilus* spp. | **Razors** *Ensis* sp. | **Pacific oysters** *Crassostrea gigas* | **Cockles** *Cerastoderma edule* | **Surf clams** *Spisula solida* |
|---|---|---|---|---|---|
| Number of samples analysed and reported | 5175 | 367 | 1173 | 335 | 65 |
| Number of samples < RL | 4927 (95.2%) | 363 (98.9%) | 887 (75.6%) | 307 (91.6%) | 32 (49.2%) |
| Number of samples AZA >RL | 248 (4.8%) | 4 (1.1%) | 286 (24.4%) | 28 (8.4%) | 33 (50.8%) |
| Number of samples ≥ ½ MPL[a] | 94 (1.8%) | 0 (0%) | 143 (12.2%) | 0 (0%) | 10 (15.4%) |
| Number of samples ≥ MPL[a] | 38 (0.7%) | 0 (0%) | 4 (0.3%) | 0 (0%) | 2 (3.1%) |
| Maximum concentration detected (µg/kg) | 626 | 26 | 237 | 34 | 209 |

[a]The number of samples ≥ ½ MPL and ≥ MPL do not add to give the total number of samples >RL. There are additional samples that produced values between the RL and ½ MPL, and some samples ≥ ½ MPL are also ≥ MPL.

Yessotoxins were only detected in mussels and no sample exceeded the current MPL of 3.75 mg/kg (Table 5.7). Several mussel samples exceeded the previous MPL of 1.0 mg/kg.

**Table 5.7:** Summary of **YTX** detections in each species commercially harvested in Scotland (July 2011 – December 2013). Data generated from LC-MS/MS testing (not mouse bioassay) is included in the table. RL = Reporting Limit. MPL = Maximum Permissible Level.

| | **Mussels** *Mytilus* spp. | **Razors** *Ensis* sp. | **Pacific oysters** *Crassostrea gigas* | **Cockles** *Cerastoderma edule* | **Surf clams** *Spisula solida* |
|---|---|---|---|---|---|
| Number of samples analysed and reported | 5175 | 367 | 1173 | 335 | 65 |
| Number of samples < RL | 4969 (96%) | 367 (100%) | 1173 (100%) | 335 (100%) | 65 (100%) |
| Number of samples YTX >RL | 206 (4.0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Number of samples ≥ ½ MPL[a] | 5 (0.1%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Number of samples ≥ MPL[a] | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Maximum concentration detected (mg/kg) | 3 | 0 | 0 | 0 | 0 |

[a]The number of samples ≥ ½ MPL and ≥ MPL do not add to give the total number of samples >RL. There are additional samples that produced values between the RL and ½ MPL, and some samples ≥ ½ MPL are also ≥ MPL.

For ASP (Table 5.8), the proportion of samples in which levels of ASP were greater than the limit of quantitation (LoQ) ranged between 4.2% for Pacific oysters to a maximum of 14.7% for surf clams. Only two samples (all species) were above the MPL (one mussel and one cockle sample) (Table 5.8).

**Table 5.8:** Summary of **ASP** detections in each species commercially harvested in Scotland (October 2010 – December 2013). LoQ = Limit of Quantitation. MPL = Maximum Permissible Level.

| | **Mussels** *Mytilus* spp. | **Razors** *Ensis* sp. | **Pacific oysters** *Crassostrea gigas* | **Cockles** *Cerastoderma edule* | **Surf clams** *Spisula solida* |
|---|---|---|---|---|---|
| Number of samples analysed and reported | 3057 | 328 | 683 | 286 | 75 |
| Number of samples < LoQ | 2912 (95.3%) | 310 (94.5%) | 654 (95.8%) | 262 (91.6%) | 64 (85.3%) |
| Number of samples ASP > LoQ | 145 (4.7%) | 18 (5.5%) | 29 (4.2%) | 24 (8.4%) | 11 (14.7%) |
| Number of samples ≥ ½ MPL[a] | 4 (0.1%) | 0 (0%) | 0 (0%) | 1 (0.3%) | 0 (0%) |
| Number of samples ≥ MPL[a] | 1 (<0.1%) | 0 (0%) | 0 (0%) | 1 (0.3%) | 0 (0%) |
| Maximum concentration detected (mg/kg) | 27 | 5.9 | 8.7 | 33 | 7.7 |

[a]The number of samples ≥ ½ MPL and ≥ MPL do not add to give the total number of samples >LoQ. There are additional samples that produced values between the LoQ and ½ MPL, and some samples ≥ ½ MPL are also ≥ MPL.

The summary statistics on all data gathered between the period October 2010 and December 2013 indicate that mussels accumulate higher concentrations of most toxins than other species of bivalves. However, it is notable that a higher proportion of surf clam samples were contaminated with PSP, DSP, AZA and ASP than mussels over the period for which data was examined. Similarly, a higher proportion of Pacific oyster samples were contaminated with AZA than mussels. These results may indicate a higher propensity for surf clams and Pacific oysters to accumulate and/or retain certain toxins than mussels, however several data limitations prohibit conclusively determining such a finding. The limitations include:

- The data presented represents all sites monitored in Scotland. Site-specific toxin events may account for differences in toxin prevalence and maximum levels between species.
- The data was gathered over a three-year period. Differences in toxin concentrations and prevalence between species may be related to the occurrence of toxin producing blooms at times during which a particular species was being intensely produced and therefore monitored, it is also possible that many samples of the same species were collected during bloom events.
- More samples of mussels and Pacific oysters were collected than the other species, potentially introducing significant bias.

Given the data limitations noted above, it is preferable to evaluate potential differences in toxin accumulation between species using data for multiple species collected from the same site on the same day.

### 5.1.3 Analysis of concurrent marine toxin data

To evaluate more closely potential differences in toxin accumulation between different bivalve species, a comparison of toxin test results between indicator and representative species (each pair as displayed in Table 5.2) was undertaken. The comparison involved examining toxin concentrations for the indicator and representative shellfish species in situations in which sample collection for both species had been undertaken in the same pod within 24 hours of each other. The comparison was undertaken using data generated

between October 2010 and December 2013 for PSP and ASP, and between July 2011 and December 2013 for DSP, AZA and YTX. Section 2 provides details on the methodology used for the comparison and the analytical methods used during the time period over which data was collected. Table 5.9 provides a summary of the indicator and representative species for which concurrent testing data were available.

**Table 5.9:** Indicator and representative species for which concurrent monitoring data (both species collected from the same pod within 24 hours of each other) are available for the time period October 2010 – December 2013.

| Indicator species | Representative species | 2010 - 2013 |
|---|---|---|
| Mussels | Pacific oysters | **Concurrent data identified** |
| | Cockles | No concurrent data |
| | King scallops | No concurrent data |
| | Native oysters | No concurrent data |
| | Razors | **Concurrent data identified** |
| Cockles | Razors | No concurrent data |
| | Pacific oysters | No concurrent data |
| Pacific oysters | Mussels | **Concurrent data identified** |
| | Queen scallops | **Concurrent data identified** |
| | Razors | **Concurrent data identified** |
| Razors | Cockles | No concurrent data |

It can be seen from this summary that limited data are available for comparing test results between species sampled from the same pod during a 24 hour period, with only four species combinations having concurrent data available:

- Mussels and Pacific oysters;
- Mussels and razors;
- Pacific oysters and Queen scallops; and
- Pacific oysters and razors.

The lack of concurrent toxin information for the other species combinations noted in Table 5.9 represents a significant data gap. For the species combinations for which concurrent monitoring data are available, simultaneous sampling was undertaken on very few occasions (always fewer than seven sampling occasions for each species combination and toxin type). The lack of data prevented formal statistical methods being used to compare the test results between species, and instead summary statistics are presented for each species combination (Tables 5.10 – 5.13). In general, on most occasions in which concurrent sampling was undertaken, both species tested had results that were less than the Reporting Limit (RL) of the test (e.g. negative test results). However, there were a few occasions in which one species showed elevated toxicity in the absence of significant toxicity in the concurrently sampled other shellfish type.

### *Comparison between mussel and Pacific oyster samples: 2010 - 2013*

Between 2010 and 2013 dual testing of mussels and Pacific oysters was undertaken on six occasions for DSP, AZA and YTX, and on four and three occasions for PSP and ASP respectively. Generally, 'not detected' results were recorded for both species. For PSP, DSP and YTX, toxins were occasionally detected in mussels, but 'not detected' in the corresponding oyster sample. No occasions were recorded in which oysters showed toxicity

alongside a corresponding absence of toxicity in mussels. Table 5.10 shows a summary of the comparative results between mussels and Pacific oysters for each toxin group.

**Table 5.10:** Summary of concurrent marine toxin testing results for the common mussel (*M. edulis*) and Pacific oyster (*C. gigas*) during the period October 2010 to December 2013. Samples of mussels and oysters were considered to be 'concurrent' if they were collected from the same pod within 24 hours of each other.

| | PSP | DSP | AZA | YTX | ASP |
|---|---|---|---|---|---|
| Occasions in which dual testing occurred | 4 | 6 | 6 | 6 | 3 |
| Occasions in which toxin 'not detected' in both species | 3 | 4 | 6 | 0 | 3 |
| Occasions in which toxin 'detected' in both species | 0 | 0 | 0 | 0 | 0 |
| Occasions in which toxins were 'detected' in oysters and 'not detected' in mussels | 0 | 0 | 0 | 0 | 0 |
| Occasions in which toxins were 'detected' in mussels and 'not detected' in oysters | 1[a] | 2[b] | 0 | 6[c] | 0 |

[a]Mussel result 'detected' but < reporting limit
[b]Mussel results were 121 and 103 µg/kg
[c]Mussel results ranged between 0.3 and 1.5 mg/kg and were related to same toxin event

### *Comparison between mussel and razor samples: 2010 - 2013*

There was only one occasion in which dual testing of mussels and razors for each of the regulated toxin groups was undertaken between October 2010 and December 2013. For PSP, AZA, YTX and ASP both species were negative, however for DSP mussels showed a level of 93 µg/kg in the absence of toxicity in razors concurrently sampled (Table 5.11).

**Table 5.11:** Summary of concurrent marine toxin testing results for the common mussel (*M. edulis*) and razors (*Ensis* sp.) during the period October 2010 to December 2013. Samples of mussels and razors were considered to be 'concurrent' if they were collected from the same pod within 24 hours of each other.

| | PSP | DSP | AZA | YTX | ASP |
|---|---|---|---|---|---|
| Occasions in which dual testing occurred | 1 | 1 | 1 | 1 | 1 |
| Occasions in which toxin 'not detected' in both species | 1 | 0 | 1 | 1 | 1 |
| Occasions in which toxin 'detected' in both species | 0 | 0 | 0 | 0 | 0 |
| Occasions in which toxins were 'detected' in razors and 'not detected' in mussels | 0 | 0 | 0 | 0 | 0 |
| Occasions in which toxins were 'detected' in mussels and 'not detected' in razors | 0 | 1[a] | 0 | 0 | 0 |

[a]Mussel result 93 µg/kg

### *Comparison between Pacific oysters and Queen scallops: 2010 - 2013*

Between 2010 and 2013 dual testing of Pacific oysters and Queen scallops was undertaken on four occasions for DSP, AZA and YTX, and on zero and three occasions for PSP and ASP respectively. Generally, toxins were not detected in either species on these sampling occasions. However, there was one sampling occasion in which DSP was detected in Queen scallops (60 µg/kg) but not in oysters, and four occasions on which YTX was detected in Queen scallops but not in oysters (Table 5.12).

**Table 5.12:** Summary of concurrent marine toxin testing results for Pacific oysters (*C. gigas*) and Queen scallops (*A. opercularis*) during the period October 2010 to December 2013. Samples of oysters and scallops were considered to be 'concurrent' if they were collected from the same pod within 24 hours of each other.

| | PSP | DSP | AZA | YTX | ASP |
|---|---|---|---|---|---|
| Occasions in which dual testing occurred | 0 | 4 | 4 | 4 | 3 |
| Occasions in which toxin 'not detected' in both species | 0 | 3 | 4 | 0 | 3 |
| Occasions in which toxin 'detected' in both species | 0 | 0 | 0 | 0 | 0 |
| Occasions in which toxins were 'detected' in oysters and 'not detected' in scallops | 0 | 0 | 0 | 0 | 0 |
| Occasions in which toxins were 'detected' in scallops and 'not detected' in oysters | 0 | 1[a] | 0 | 4[b] | 0 |

[a]Queen scallop result 60 µg/kg

[b]Queen scallop results ranged between 1.4 and 1.5 mg/kg. All four sampling occasions were undertaken over a 4-week period and likely related to the same toxic event.

### *Comparison between Pacific oysters and razors: 2010 - 2013*

There were only two occasions on which dual testing of Pacific oysters and razors were undertaken for each of the regulated toxin groups. Toxins were not detected in either species on both sampling occasions (Table 5.13).

**Table 5.13.** Summary of concurrent marine toxin testing results for the Pacific oyster (*C. gigas*) and razors (*Ensis* sp.) during the period October 2010 to December 2013. Samples of oysters and razors were considered to be 'concurrent' if they were collected from the same pod within 24 hours of each other.

| | **PSP** | **DSP** | **AZA** | **YTX** | **ASP** |
|---|---|---|---|---|---|
| Occasions in which dual testing occurred | 2 | 2 | 2 | 2 | 2 |
| Occasions in which toxin 'not detected' in both species | 2 | 2 | 2 | 2 | 2 |
| Occasions in which toxin 'detected' in both species | 0 | 0 | 0 | 0 | 0 |
| Occasions in which toxins were 'detected' in oysters and 'not detected' in razors | 0 | 0 | 0 | 0 | 0 |
| Occasions in which toxins were 'detected' in razors and 'not detected' in oysters | 0 | 0 | 0 | 0 | 0 |

### *Summary of comparative results for ASP and PSP in mussels and King scallops: 2001 - 2008*

The literature review component of this report identifies a potential higher propensity for the accumulation of ASP and PSP toxins by King scallops compared to mussels. Due to the lack of data for King scallops in the period 2010 – 2013, a comparison of ASP and PSP concentrations in mussels and King scallops was undertaken using historical data generated between 2001 and 2008. Samples of scallops and mussels were considered to be concurrent if sample collection for both species had been undertaken in the same pod within 36 hours of each other. During this period mouse bioassay testing was undertaken for the PSP toxins and an HPLC method was used for the ASP toxins (Howard, 2002). DSP was not considered in this analysis due to the lack of information regarding the definitive identification of the lipophilic toxin(s) responsible for a DSP positive result.

For PSP, concurrent samples of scallops and mussels were collected on 19 occasions. On 10 of these occasions both the scallop gonad and whole scallop tissue were tested, on the other nine occasions either the scallop gonad or whole scallop were tested. While the entire historical dataset was scrutinised, all concurrent scallop and mussel samples were collected in 2001 and 2002. PSP toxins were not detected in either scallop or mussel samples on each of the 19 concurrent sampling occasions (Table 5.14).

**Table 5.14:** Summary of concurrent marine toxin testing results for mussels (*M. edulis*) and King scallops (*P. maximus)* during the period 2001 to 2008. Samples of mussels and scallops were considered to be 'concurrent' if they were collected from the same pod within 36 hours of each other.

| | PSP | ASP |
|---|---|---|
| Occasions in which dual testing occurred | 19 | 3 |
| Occasions in which toxin 'not detected' in both species | 19 | 0 |
| Occasions in which toxin 'detected' in both species | 0 | 0 |
| Occasions in which toxins were 'detected' in mussels and 'not detected' in King scallops | 0 | 0 |
| Occasions in which toxins were 'detected' in King scallops and 'not detected' in mussels | 0 | 3 |

For ASP, concurrent samples of scallops and mussels were collected on three occasions in 2001. On each of the three sampling occasions ASP toxins were detected in scallop gonad tissue but not in concurrently collected mussels (Table 5.14). On one occasion whole scallop tissue was also tested and found to have a level of 25 mg/kg, scallop gonad tissue had a concentration of 3 mg/kg, whereas ASP was not detected in mussels concurrently collected (Table 5.15).

**Table 5.15:** ASP test results for concurrently collected samples of mussels (*M. edulis*) and King scallops (*P. maximus*) during the period 2001 to 2008.

| Pod Number | Date | Mussel | Scallop gonad | Whole scallop |
|---|---|---|---|---|
| 36 | 14 – 15 May 2001 | Not detected | 3 | 25 |
| 36 | 30 July – 2 August 2001 | Limit of detection[a] | 5 | NT |
| 42 | 19 – 20 September 2001 | Limit of detection[a] | 12 | NT |

[a]Database notes the result as 'Limit of detection'. It is not clear if this represents a positive result at around the limit of detection of the test, or a 'not detected' result.

### *Summary of comparative results for mussels and Pacific oysters: 2001 - 2008*

In 2008 an update of the 2006 risk assessment on marine toxins in Scotland was undertaken. As part of the updated risk assessment it was noted that "it became apparent that there was often a discrepancy in the levels of PSP and DSP observed between mussels and Pacific oysters, with the latter often having lower levels (or absence) of the toxin than the corresponding mussel samples" (Holtrop, 2008). Given this observation Holtrop (2008) investigated comparative levels of DSP and PSP between 2001 and 2008.

It was noted that nine of the 806 Pacific oysters tested between April 2001 and March 2008 were positive for DSP and that two of the 1088 oyster samples tested positive for PSP. Due to the low number of positives no formal statistical analysis was undertaken. Key findings of the qualitative analysis included:

- For DSP, between 2001 and 2008 it was noted that there were 163 instances in which Pacific oysters and mussels were sampled and tested from the same pod during the same week. During this period there were two instances in which Pacific oysters were positive and mussels were negative, and 22 instances in which oysters were negative and mussels were positive. There were 138 occasions on which both species were negative.
- For PSP, between 2001 and 2008, it was noted that there were 157 instances in which Pacific oyster and mussels were sampled and tested from the same pod during the same week. During this time there was one instance in which oysters

were positive and mussels were negative and two instances in which oysters were negative and mussels were positive. There were 154 occasions in which both species were negative.

Due to data limitations and the low numbers of positive results, the authors further compared mussel and oyster results for groups of pods that are geographically close to each other. Based on this wider analysis, the authors noted that while mussels were positive for DSP and PSP more frequently than Pacific oysters, when a positive result was recorded in oysters "more often than not (in seven out of 11 cases) this coincided with a negative result in mussels from the corresponding group of pods, for up to four weeks prior to the Pacific oyster sample being taken". The authors concluded that using mussels as an indicator species for both PSP and DSP may not be satisfactory (Holtrop, 2008). However, algal blooms can be very localised to small geographic regions (Bricelj and Shumway, 1998) and it is not clear whether the intraspecies differences in toxin accumulation noted in the wider analysis based on groups of pods reflects geographical differences in algae and toxin occurrence (and thus differences in exposure of the two species), or true differences in accumulation between similarly exposed species.

For DSP, it is noteworthy to mention that no information was provided regarding which of the lipophilic toxins (OA, DTX, PTX, YTX or AZA) were responsible for the toxicity in mussels and oysters, in either the within pod analysis or the wider analysis considering groups of pods. This is likely due to the implementation of the DSP mouse bioassay over the period 2001 – 2010. Conclusions are difficult to reach regarding differences in accumulation of the lipophilic toxins (synonymous with DSP toxins over this period) between oysters and mussels during this time, as it is not known what toxins were responsible for DSP positive results.

### 5.1.4 Summary of data analysis

Based on the analysis of all toxin data generated between October 2010 and December 2013, mussels were found to accumulate the highest concentration of each toxin group (except ASP) compared to other commercial bivalve species tested over this period of time. A higher proportion of surf clam samples contained PSP, DSP and AZA compared to mussels, and similarly a higher proportion of oyster samples contained AZA than mussel samples.

To investigate these results further the data was scrutinised for occasions in which indicator and representative species had been concurrently sampled and tested for toxins. In general very limited data were identified which had been generated for two species located in the same area and sampled within 24 hours of each other. Concurrent data from 2010 to 2013 were only identified for the following combinations of species:

- Mussels and Pacific oysters;
- Mussels and razors;
- Pacific oysters and Queen scallops; and
- Pacific oysters and razors.

Most of the concurrent data identified for these species combinations showed results for both species that were below the LoD of the tests undertaken (i.e. 'negative'). This limits conclusions that can be drawn. However, comparative results over the 2010 to 2013 period suggest that mussels more readily accumulate YTX than oysters. Additionally, the results showed 10 occasions on which toxins (PSP, DSP and YTX) were detected in mussels in the absence of toxicity in concurrently collected Pacific oysters and razors.

Further analysis of an historical database with toxin results collected between 2001 and 2010 was undertaken. For King scallops historical data demonstrated that ASPs accumulate to higher concentrations in the scallop gonad and whole scallop tissues, compared to mussels concurrently sampled.

# SECTION SIX: SUMMARY OF DATA GAPS

Key data gaps that are relevant to the situation in Scotland that have been highlighted through the literature review (Section 3) and the analysis of Scotland's monitoring data (Section 5) are summarised below.

## 6.1 Marine toxins

1. Some published data regarding toxin accumulation exists from concurrent monitoring of Pacific oysters and common mussels for each of the toxin groups of regulatory concern. Some published data also exists regarding comparative accumulation of DSP, PSP and ASP in King scallops and common mussels, and between various clam species and mussels. There is a paucity of published data however, regarding comparative accumulation of all regulated toxin groups by razors (*Ensis* sp.), cockles (*C. edule*), native oysters (*O. edulis*) and Queen scallops (*A. opercularis*).

2. There is no concurrent monitoring data available in Scotland for many indicator/representative species combinations currently used in support of the official control biotoxin programme. Table 5.9 provides a summary for species for which data is not available. Limited amounts of concurrent monitoring data are available for:
   - Mussels and Pacific oysters;
   - Mussels and razors;
   - Pacific oysters and Queen scallops;
   - Pacific oysters and razors; and
   - King scallops and mussels.

   While some concurrent data exists for these species, generally the data shows 'not detected' results for both species tested. Thus data is lacking regarding comparative accumulation during toxin producing algal blooms.

3. There is an indication that comparatively high levels of PSPs can accumulate in scallops compared with mussels (Lassus et al., 1989; Oshima et al., 1982). An analysis of Scotland's monitoring data reveals 19 occasions in which concurrent monitoring of PSP has been undertaken in King scallops and mussels. However, no detections were observed for either species, thus no useful information is available to assess the appropriateness of mussels as an indicator for King scallops. There are currently two pods in Scotland in which the common mussel is used as an indicator species for King scallops.

4. For ASP, there is evidence from the published literature that clams (*D. trunculus*) accumulate higher concentrations than mussels (*M. edulis/galloprovincialis*). Additionally, analysis of Scotland's monitoring data reveals that a higher proportion of surf clam (*S. solida)* samples contained PSP, DSP and AZA than other commercially produced bivalve species. There is currently one pod in Scotland in which mussels may periodically represent surf clams (when clams are not able to be accessed by samplers). It is unclear however, whether surf clams have the potential to accumulate elevated levels of ASP and other toxins more rapidly than the common mussel or other bivalves (i.e. there is no concurrent monitoring data to evaluate this).

5. Data from Ireland suggests that Pacific oysters are efficient accumulators of AZA and may at times contain higher concentrations than the common mussel. It is unclear whether the data relates to more efficient accumulation of AZA by oysters, or to slower

elimination rates of AZA by oysters than mussels (or a combination of both processes) and there is a lack of comparative data for oysters and mussels during AZA events in Scotland.

6. While some comparative data regarding accumulation of toxins does exist (as noted above), published studies have not generally noted at what point in the progression of a bloom samples were collected, but rather report the maxima recorded during a bloom event for each species. This approach means it is difficult to assess relative concentrations between species in the uptake phase of the bloom versus the elimination phase of the bloom.

7. Several published studies indicate that clams (*R. philippinarum* and *D. trunculus*) eliminate DSP and PSP at slower rates than mussels (*M. edulis* and *M. galloprovincialis*). Cockles have also been classified as a slow detoxifier of PSP compared with mussels. There are limited data regarding the comparative elimination of marine toxins between species of clams and cockles commonly found in Scotland (i.e. *S. solida* and *C. edule*) and the common mussel.

8. Information obtained from the literature and other countries that use shellfish indicator species for marine toxin management indicates that toxins (toxin type not specified) may be eliminated at slower rates from oysters and scallops than from mussels. Limited data exist regarding comparative elimination of toxins by these species.

## 6.2 Chemical contaminants

1. For the metals, significant published information is available regarding accumulation and retention of Cd in oysters compared to mussels. Two studies, one on Hg and the other on Pb, found higher concentrations in mussels compared to co-sampled oysters, however comparative data regarding uptake of these metals by multiple species of bivalves in the natural environment is scant.

2. While several investigations have focused on comparative uptake of metals by oysters and mussels, few have involved concurrent monitoring of contaminants in other bivalve species of interest in Scotland e.g. scallops, surf clams, razors and cockles.

3. One study found higher levels of PAHs and PCBs in oysters (*Crassostrea* sp.) compared to co-located mussels (*M. galloprovincialis*), however data is limited regarding relative accumulation of PAHs, dioxins and DL-PCBs in commercial species of relevance to Scotland.

4. There is a lack of data regarding contaminant elimination by different bivalve species that are co-located and exposed to the same environmental conditions.

5. Data is limited regarding the concentration of chemical contaminants in different co-located bivalve species, and the potential relationship/association to variation in exposure patterns, which may be related to differences in the bivalve's habitats.

# SECTION SEVEN: DISCUSSION AND CONCLUSIONS

## 7.1 Marine toxins

Bivalve shellfish readily accumulate marine toxins, primarily through the process of filter feeding on toxin producing phytoplankton. Some species of bivalves accumulate particular types of marine toxins more efficiently than other species, for example mussels more readily accumulate PSP and DSP toxins than oysters that co-occur in the same location. There are several reasons for differences in accumulation of toxins between bivalve species. The major causes relate to significant variations in feeding and filtration physiology between bivalves, and differences in the degree of exposure of different shellfish species due to variations in the marine habitats that each bivalve occupies.

Published literature demonstrates that mussels generally appear to accumulate higher concentrations of marine toxins than most other bivalve species when they are co-located, making them an ideal candidate as a shellfish indicator species in most circumstances, however, the reviewed literature also highlights several exceptions which are discussed in more detail below. An analysis of comparative data generated in Scotland between 2010 and 2013 found 10 occasions on which toxins (PSP, DSP and YTX) were detected in mussels in the absence of toxicity in concurrently collected Pacific oysters and razors (no comparative data exist for mussels and other bivalve species over this period). Conversely, there were no occasions in which Pacific oysters or razors showed toxicity in the absence of toxins in mussels. Consistent with this finding, official control programme data between 2010 and 2013 demonstrates that mussels have accumulated higher concentrations of most toxins (with the exception of ASP) than other species of bivalves in Scotland (Section 5.1.2). Together these findings support the conclusion of the literature review regarding the general utility of mussels as an indicator species. However, it is emphasised that there are very few comparative data available with which to draw firm conclusions regarding relative accumulation and detoxification between species.

Data presented in a series of publications regarding AZA contamination of shellfish in Ireland demonstrate that oysters efficiently accumulate AZA and may at times have higher concentrations of AZA than co-occurring mussels. Azaspiracid has been monitored separately in the official control programme in Scotland since July 2011, since this time there have been six occasions in which samples of mussels and Pacific oysters were concurrently sampled and tested for AZA (Table 5.10). Unfortunately AZA was not detected in either species on any of the six occasions. Between 2011 and 2013 AZA was detected in a higher proportion of oyster samples than mussel samples: 24.4% of oyster samples contained AZA, whereas 4.8% of mussel samples contained AZA. Similarly, the proportion of oyster samples exceeding the level of 80 µg/kg (1/2 MPL) was higher than the proportion of mussel samples (12.2% vs. 1.8%). Consistent with the findings of the literature review, the higher proportion of oyster samples containing AZA in Scotland may indicate an increased propensity for oysters to accumulate AZA as compared to mussels. Differences in geographical location of mussels (grown sub-tidally on long lines) and oysters (grown in the inter-tidal zone), and variance in depuration rates between species may also contribute to the higher proportion of AZA positive oysters than mussels in Scotland. However, the lack of comparative data for oysters and mussels during AZA events prevents firm conclusions regarding comparative accumulation and elimination of AZA by these shellfish species.

Conflicting results were noted in the published literature regarding the relative accumulation of YTX by mussels and oysters, one laboratory study noted higher concentrations of YTX in oysters compared with mussels (Röder et al., 2011), whereas

several field studies have recorded higher levels of YTX in mussels than oysters (Amzil et al., 2008; Gomes et al., 2006; Vale et al., 2008b). The comparative results obtained in the official control programme since monitoring of YTX as a separate toxin group commenced shows six occasions on which oysters and mussels were concurrently sampled from the same pod at the same time. These results show that on all six occasions mussels had detectable levels of YTX, while YTX was not detected in oysters that were co-sampled (Table 5.10). This supports the findings that mussels accumulate YTX more readily than oysters in field situations.

Published data from several separate studies suggests that King scallops can accumulate higher concentrations of ASP and PSP than co-occurring mussels. Unfortunately no data was gathered for King scallops grown in the in-shore environment as part of the official control programme in Scotland during the period October 2010 to December 2013. However, analysis of historical data gathered between 2001 and 2010 for ASP in King scallops and mussels revealed three occasions on which concurrent samples were collected; on each occasion scallops contained elevated levels of ASP, whereas mussels did not contain detectable concentrations. On each of these occasions ASP concentrations were detected in the gonad, and on one occasion whole scallop tissue was also tested and found to contain 25 mg/kg ASP (most of which was likely to be localised in the digestive tissue). The historical data also revealed 19 occasions on which both species were analysed for PSP toxins, however no toxins were detected. The historical data supports the findings of the literature review with respect to King scallops having a higher propensity to accumulate ASP toxins than mussels. However the findings regarding PSP are inconclusive as concurrent King scallop and mussel samples do not appear to have been gathered during PSP producing algal blooms.

With respect to toxin elimination, it is clear that depuration rates of toxins differ significantly between bivalve species. The elimination of DSP from two clam species is suggested to be slower than mussels. For PSP, mussels, oysters and scallops have been classified as 'fast detoxifiers', whereas the cockle (*C. edule*) is considered a slow detoxifier. Consistent with this PTXs have been shown to depurate faster in mussels than cockles. Several countries also identified slow depuration of toxins (unspecified) by oysters and scallops relative to mussels as a potential issue in using indicator species for management purposes. Supporting this, one study demonstrated slower elimination of PSP from the scallop *P. yessoensis* compared to the mussel *M. edulis* (Oshima et al., 1982). The reasons for variation in toxin elimination rates between bivalve species is generally not well understood, however it seems likely that this relates to a range of factors, such as: the rate of defaecation and excretion of toxins, the conversion of the toxins from one toxin analogue to another, and the degradation of the toxins to nontoxic compounds within the bivalve. Few data exist from the official control monitioring programme with which to evaluate relative toxin elimination by different bivalve species in Scotland.

Table 7.1 shows the current indicator species used in the Scottish marine toxin official control programme, and the species represented by the indicator. Table 7.1 also provides a summary of concurrent monitoring data that has been generated in Scotland during toxin producing blooms since the introduction of HPLC for PSP and LC-MS/MS testing for DSP in 2010 and 2011 respectively. It can be seen from this summary that only four species combinations used in Scotland have concurrent data available:

- Mussels and Pacific oysters;
- Mussels and razors;
- Pacific oysters and Queen scallops; and
- Pacific oysters and razors.

For the species combinations for which concurrent monitoring data are available, simultaneous sampling was undertaken on very few occasions during toxin producing bloom events (always fewer than seven sampling occasions for each species combination and toxin type). The only toxin group for which sufficient Scottish data is available to support the choice of an appropriate indicator species is YTX, for the species combinations: mussels and Pacific oysters, and Pacific oysters and Queen scallops. Fewer than two data points exist for all other species/toxin combinations.

**Table 7.1:** Indicator and representative species for which concurrent monitoring data (both species collected from the same pod within 24 hours of each other) are available for the time period October 2010 – December 2013.

| Indicator species | Representative species | 2010 - 2013 | Number of concurrent sampling occasions during blooms | | | | |
|---|---|---|---|---|---|---|---|
| | | | PSP | DSP | AZA | YTX | ASP |
| Mussels | Pacific oysters | **Concurrent data identified** | **1** | **2** | **0** | **6** | **0** |
| | Cockles | No concurrent data | | | | | |
| | King scallops | No concurrent data | | | | | |
| | Native oysters | No concurrent data | | | | | |
| | Razors | **Concurrent data identified** | **0** | **1** | **0** | **0** | **0** |
| Cockles | Razors | No concurrent data | | | | | |
| | Pacific oysters | No concurrent data | | | | | |
| Pacific oysters | Mussels | **Concurrent data identified** | **1** | **2** | **0** | **6** | **0** |
| | Queen scallops | **Concurrent data identified** | **0** | **1** | **0** | **4** | **0** |
| | Razors | **Concurrent data identified** | **0** | **0** | **0** | **0** | **0** |
| Razors | Cockles | No concurrent data | | | | | |

The lack of concurrent toxin information for species combinations of relevance to the Scottish situation represents a significant data gap. Recommendations regarding future research that could be undertaken to improve the knowledge base regarding appropriate indicator species to use during the accumulation and elimination phase of toxic events are provided for consideration in Section 8.

## 7.2 Chemical contaminants

Bivalve shellfish accumulate organic (i.e. PAHs, dioxins and DL-PCBs) and inorganic contaminants (i.e. metals) that may be present in the marine environment via two mechanisms: direct absorption across the gills and through the process of filter feeding and consumption of particulate matter to which the contaminants are bound. While there is a wealth of data reporting concentrations of various chemical contaminants in bivalve shellfish, the literature base is relatively limited regarding studies in which accumulation and elimination of the regulated chemical contaminants have been determined in different species of bivalves that have been concurrently sampled from the same location at the same time.

However, some published literature is available and with respect to Cd, the results of this review suggest that oysters accumulate higher concentrations than mussels and clams. There is also some evidence that suggests that scallops accumulate high concentrations of Cd. While there is significant published data pertaining to the accumulation of Cd in oysters compared to mussels, comparative data regarding accumulation of Hg, Pb and the organic contaminants is scant. Few studies involving side-by-side monitoring of contaminants in

multiple bivalve species have included the other commercial species of interest in Scotland e.g. scallops, surf clams and cockles.

With respect to contaminant elimination, there are large apparent differences in elimination timeframes (biological half-life) for contaminants by different shellfish species. In general, however, these data were generated in separate studies of bivalves maintained in different environmental conditions and thus comparisons of elimination rates between species are hampered. The lack of such data hinders analysis regarding the appropriate bivalve species to use as an indicator of contamination following environmental contamination events and in routine type monitoring programmes – the obvious choice being a species that exhibits prolonged biological half-life values.

It is noted that in Scotland indicator shellfish species are not currently used in the risk management programme for chemical contaminants in shellfish. Of the 12 countries that returned responses to the shellfish indicator survey undertaken as part of this review, only two countries noted that they use indicator species (mussels) for chemical contaminants. In Scotland (and other countries) the risk management programme up to 2014 has involved the *ad hoc* collection of a single bivalve species (species type not mandated) from a selection of different production areas once per annum for testing of the regulated contaminants[11]. In this manner, it is considered by the FSA that by 2014 a baseline assessment of contaminant levels of all Scottish production areas has been achieved. In general it appears that multiple species have not been gathered concurrently from the same location at the same time (Fernandes et al., 2013). The way in which the data has been gathered prevents meaningful analysis of comparative accumulation between different species. This is consistent with the view of Fernandes et al. (2013) who note in the 2013 summary report for the FSA on chemical contaminant sampling and analysis of shellfish from classified harvesting areas that "it would be inappropriate to compare concentrations across the species".

The lack of both published and routine monitoring data regarding comparative accumulation and elimination means that it is difficult to form robust conclusions regarding the relative sensitivity of bivalve species to the various regulated contaminants. Several recommendations are made (Section 8) regarding future data collection that may enable meaningful between species comparisons to be made in the future and support discussions on the potential use of indicator species for contaminants in risk management programmes.

[11]From 2014 the Scottish management programme involves the collection of 1 sample per annum from 30 areas for testing Cd, Hg, Pb and PAHs, and the collection of 1 sample per annum from 5 out of the 30 areas that are considered to be at higher risk of contamination for Cd, Hg, Pb, PAHs, dioxins and DL-PCBs.

# SECTION EIGHT: RECOMMENDATIONS ON FUTURE RESEARCH

The data gaps identified from the literature review and analysis of monitoring data have been used as a basis to develop a series of recommendations on potential future research. The recommendations are presented in order of priority. These priorities have been formed based on the relative importance of the shellfish species concerned (in terms of volumes produced and hence consumed), and the comparative public health risk of a particular toxin or contaminant.

## 8.1 Marine toxins

1. Marine toxin data generated from concurrent sampling of indicator and representative species commonly used in Scotland are limited. While some concurrent monitoring has occurred, generally this has been undertaken at times when toxin is not present in the area, resulting in 'negative' results in both species sampled. In Scotland, mussels are most commonly used as an indicator species for Pacific oysters (12 pods) and cockles (seven pods). Cockles are also used as an indicator species for razors in three pods (Table 5.2). It is recommended that when algal blooms occur in pods in which these particular species co-occur (i.e. mussels and Pacific oysters; mussels and cockles; and cockles and razors) that dual monitoring of these species is undertaken throughout and following the bloom events. It is suggested that dual monitoring be undertaken while the bloom is active (i.e. in the accumulation phase) and following the termination of the bloom (i.e. in the toxin depuration phase). Some shellfish may accumulate toxin rapidly and thus be a useful indicator during the active bloom period, but also rapidly depurate the toxin prior to other species and therefore may not be a useful indicator of shellfish safety following bloom termination. Such an approach should enable more meaningful conclusions to be reached regarding the appropriateness of these important indicator species that are commonly used in Scotland. Similar monitoring could also be undertaken to strengthen the dataset for other indicator/representative species combinations used in Scotland. However, the species proposed above are the most significant in terms of current use as indicators in the programme, and in terms of volumes of shellfish produced and consequently consumed (mussels and razors are the most significant species by volume and value in Scotland).

2. As noted above, it is recommended that dual samples be collected during the toxin depuration phase following termination of algal blooms. This may be particularly important for cockles, which are noted to be 'slow detoxifiers' and thus may retain toxins for a longer period than mussels. It is noted that currently indicator species are used in Scotland both prior to and following bloom events. Most other countries surveyed as part of this project test individual species when considering re-opening areas to ensure all species in an area are below the toxin MPL. The collection of data during the toxin elimination phase should provide information with which to inform risk management decisions regarding the use of indicator species for re-opening purposes following toxin events.

3. The literature review found that Pacific oysters efficiently accumulate AZA and may at times have higher concentrations than co-occurring mussels. Scotland's monitoring data also shows that a higher proportion of oyster samples contained AZA as compared to mussels. However, there is a lack of comparative data for Pacific oysters and mussels during AZA events in Scotland, which prevents firm conclusions regarding comparative accumulation and elimination of AZA by these species. To assist further appraisal of

appropriate indicator species for AZA, it is recommended that concurrent monitoring of mussels and Pacific oysters is undertaken (as noted in recommendation 1), preferably at times when AZA-producing organisms and AZA are present in the water column.

4. Published literature and data from the official control programme demonstrate elevated levels of ASP in King scallop gonad and whole scallop tissue (presumably including the gut) relative to co-located mussels (both species located in the in-shore environment). It is recognised that scallops are generally sold as 'meat' only products that likely pose a much lower biotoxin risk than products containing gonad (or roe) or gut. To verify this assumption and to evaluate if mussels provide an adequate indication of ASP risk in King scallop meat, it is suggested that dual sampling of scallop meat and co-located mussels be undertaken during an ASP-producing *Pseudo-nitzschia* bloom.

5. There is an indication from the literature that King scallops may accumulate higher concentrations of PSP than co-occurring mussels, however no data is available from the in-shore toxin monitoring programme to evaluate comparative uptake during PSP-producing blooms. Given the current use of mussels as an indicator organism for King scallops, it is recommended that dual monitoring of both shellfish species is undertaken, preferably during PSP-producing algal blooms. It can be difficult to predict when such blooms will occur and thus sometimes the opportunity to sample both species during blooms can be missed, one approach to overcome this issue is to re-locate a small volume of live mussels and scallops to an area in which recurrent PSP blooms are known to occur for targeted sampling during an active PSP event.

6. It is noted that between 2010 and 2013 a higher proportion of surf clam samples were found to contain PSP, DSP and AZA than other bivalve species commercially produced in Scotland, and there is some evidence from the literature that ASP may be more efficiently accumulated by *Donax* species of clams than mussels. Currently there is only one pod that is classified for surf clams in Scotland, mussels may be used to periodically represent surf clams (when clams are not able to be accessed by samplers); – however this does not appear to have been enacted between 2010 and 2013. It is suggested that if an indicator species is to be used in the future for surf clams, that some verification sampling is undertaken during and following toxin producing algal bloom events to ensure that the indicator species is equally, or more, sensitive than surf clams for the various toxin groups.

7. Comparisons of the accumulation of the lipophilic toxins between species during the period 2001 – 2011 are difficult to make as the mouse bioassay was used for so called 'DSP' toxins over this period. This means it is not possible to ascertain which lipophilic toxins were responsible for a positive DSP result and limits the value of any comparison. Since 2011, LC-MS/MS has been used for the lipophilic toxins and currently toxins are reported in the database (which holds official control data) as follows:
    a. DSP total (which comprises congeners of OA, DTX and PTX summed together)
    b. YTX
    c. AZA

    In the future it may be desirable to evaluate comparative accumulation of OA, DTX and PTX toxin congeners separately – as there is some evidence of differential accumulation and elimination of these congeners by bivalve species (Vale, 2004a, 2006). Thus it is recommended that the current reporting system for the DSP group (OA, DTX and PTX) be changed to incorporate separate fields for the OA, DTX and PTX congeners into the database, along with the current 'total DSP' field.

8. The questionnaire distributed to a range of shellfish producing countries as part of this project (Section 4) identified five countries that have unpublished concurrent monitoring data, which is purported to support the use of indicator species. Consideration could be given to requesting access to these unpublished datasets and undertaking further data analysis to evaluate the effectiveness of indicator species. This may assist in addressing some of the data gaps identified as part of this project.

## 8.2 Chemical contaminants

1. This review suggests that oysters accumulate higher concentrations of Cd than mussels and clams, however comparative data regarding accumulation of Hg, Pb and the organic contaminants in different bivalve species is scant. While some data is available for mussels and oysters, few studies involving dual monitoring of contaminants in multiple bivalve species have included the other commercial species of interest in Scotland e.g. scallops, surf clams and cockles. Currently indicator species are not used in Scotland as part of the management programme for chemical contaminants. Given this, the monitoring programme implemented between 2006 and 2013 has focused on the analysis of one bivalve species per site and does not enable meaningful comparisons of contaminant levels between species. To inform future deliberations regarding the potential adoption of an indicator approach, similar to that used for toxin management (and to support the choice of appropriate indicator species), concurrent monitoring data would be required. If such an approach is taken it is recommended that a statistically based sampling programme be designed and implemented which involves sampling multiple bivalve species at the same time from each of several different monitoring sites. It is recommended that any sampling programme, such as this, accounts for the potential for seasonal fluctuation in contaminant accumulation in different bivalve species.

2. Currently chemical contaminant data for metals, PAHs, dioxins and DL-PCBs that were derived over the period 2006 to the current time are held in a series of excel files. The excel files are not formatted in such a way as to enable the data to be easily scrutinised, for data queries to be run, or for easy export into statistical analysis programmes or relational databases. Given (a) the changes in the contaminant-monitoring programme in 2014 and need for a repository for new data generated, and (b) the potential need for future data analysis to support policy decisions, it is recommended that the historical data is collated into a central database that can be used on an on-going basis. For cost-saving measures this could be a simple database system such as Microsoft access, or an appropriately formatted spread sheet such as an Microsoft excel file that includes separate fields for the following parameters:
    - Date of sampling
    - Date of analysis
    - Pod sampled
    - Production area sampled
    - Species sampled
    - Name of contaminant congener
    - Regulated contaminant total

    It is also recommended that the database include individual contaminant congeners, but also clearly identifies the regulated contaminant totals.

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# Appendix One: Evaluation of key publications considered in the literature review

An evaluation of the most relevant papers considered in the literature review was undertaken (Table A1). The scientific findings of each paper are discussed in the literature review section of this report (Section 3). The methodology (Section 2) provides details on the selection process for publications included in the evaluation and the approach used to evaluate the papers. Briefly, the selected papers were critiqued against a series of pre-determined questions. The questions used to evaluate the papers were:

**Question 1:** Were appropriate analytical test methodologies used for toxins and contaminants?
**Question 2:** Were the different bivalve species located in the same production area, or within close proximity of each other?
**Question 3:** Were samples of different species collected from the same site at the same time?
**Question 4:** Was the number of field sites included in the study sufficient to support generalisations regarding relative accumulation and elimination by different bivalve species?
**Question 5:** Were the number of collection events and/or samples analysed sufficient to support generalisations regarding relative accumulation and elimination by different bivalve species?
**Question 6:** Did the study design, data and statistical treatment support the conclusions?

For each of the papers considered, the questions above were assessed and a score of 0 (no), 1 (acceptable/generally) or 2 (yes) was allocated for each question. A total score was calculated for each paper, thus high scoring papers are suggestive of robust results and conclusions (a maximum score of 12 is possible). Tables A1 and A2 provide the results for the evaluation.

Table A1. Summary of evaluation undertaken for key marine toxin publications considered in the literature review

| | **Q1:** Appropriate methods used? | **Q2:** Bivalve species co-located? | **Q3:** Were samples collected at the same time? | **Q4:** Number of field sites sufficient? | **Q5:** Number of collection events and samples sufficient? | **Q6:** Study design appropriate to support conclusions? | **TOTAL SCORE** |
|---|---|---|---|---|---|---|---|
| **Marine toxin publications** | | | | | | | |
| Oshima et al. (1982) | **2** (AOAC MBA) | **2** (yes) | **2** (yes) | **2** (study evaluated concurrent data from 7 different sites) | **2** (concurrent samples were collected on 10 occasions during a bloom) | **2** (yes) | **12** |
| James et al. (2005) | **2** (LC-UV) | **1** (information provided is limited) | **1** (information provided is limited) | **1** (1 example of concurrent monitoring at 1 site noted) | **0** (not clear at what point in the bloom concurrent samples were collected, or if multiple samples were collected through a bloom) | **0** (lack of cited concurrent data means it is difficult to assess if conclusions are appropriate) | **5** |
| Amzil et al. (2001) | **2** (LC-DAD) | **1** (information provided is limited) | **1** (information provided is limited) | **1** (relates to 1 event in 1 area) | **0** (not clear if sampling of 2 species was concurrent or how many concurrent collection events were undertaken, therefore generalisations not possible) | **2** (conclusions did not draw comparisons between toxin levels in species and were appropriate to data collected) | **7** |
| Furey et al. (2003) | **2** (LC-MS/MS) | **2** (yes, concurrent sample collected from County Donegal) | **2** (yes) | **1** (concurrent data presented only relates to 1 site) | **0** (not clear at what point in the bloom concurrent samples were collected, or if multiple samples were collected through a bloom) | **2** (conclusions regarding interspecies differences in toxin levels were appropriate) | **9** |

Table A2. Summary of evaluation undertaken for key contaminant publications considered in the literature review

| | **Q1:** Appropriate methods used? | **Q2:** Bivalve species co-located? | **Q3:** Were samples collected at the same time? | **Q4:** Number of field sites sufficient? | **Q5:** Number of collection events and samples sufficient? | **Q6:** Study design appropriate to support conclusions? | **TOTAL SCORE** |
|---|---|---|---|---|---|---|---|
| **Chemical contaminant publications** | | | | | | | |
| Fang et al. (2003) | **2** (Spectroscopy, included use of certified standards) | **2** (yes, all species sampled at 5 sites) | **2** (all samples collected in July and August 1996) | **2** (evaluated concurrent data from 5 different sites, and from a further 20 sites) | **1** (collection occurred over a short time frame, 2 months. Conclusions would be better supported if further collection events occurred at additional time points) | **1** (conclusions regarding differences between species would be better supported if additional collection events at different times of the year were undertaken) | **10** |
| Rojas de Astudillo et al. (2005) | **2** (Spectroscopy, included use of certified standards) | **2** (yes, concurrent collection at 6 sites) | **2** (all samples collected in same month) | **2 (**6 different field sites concurrently sampled, and several additional sites in which 1 species was evaluated. | **1** (collection occurred over 1 month. Conclusions would be better supported if further collection events occurred at additional time points) | **1** (conclusions regarding differences between species would be better supported if additional collection events at different times of the year were undertaken) | **10** |
| Martincié et al. (1984) | **1** (Spectroscopy based method used, not clear if certified standards used) | **2** (bivalves suspended in cages in same place in water column) | **2** (yes) | **1** (1 field site for bivalves was evaluated) | **1** (a single sample collection event for bivalves was undertaken) | **2** (conclusions regarding interspecies differences in toxin levels were appropriate) | **9** |
| Law et al. (1999) | **2** (GC-MS used with standards as available) | **1** (yes, but no details of location provided) | **1** (yes, but no details of time of sampling provided) | **1** (unclear, as no details on concurrent samples were provided) | **1** (unclear, as no details on concurrent samples were provided) | **2** (conclusions did not draw comparisons between toxin levels in species and were appropriate to data collected) | **8** |
| Orbea et al. (2002) | **2** (GC-MS used with standards) | **2** (yes, oysters and mussels co-located at 2 sites) | **2** (yes, 2 sampling periods, summer and winter) | **1** (2 field sites concurrently sampled) | **1** (use of 2 sampling periods in winter and summer appropriate and supports generalisation, however the number of study sites and samples collected are limited) | **2** (yes) | **10** |

## Appendix Two: Survey on shellfish indicator species used in international risk management programmes

1) **Does your country have production areas in which cultivation or harvesting of multiple shellfish species occurs within the same area?**
   Yes: ☐
   No: ☐
   Please insert comments here:

*If you answered 'yes' to question 1, please answer questions 2 - 17. If you answered 'no', please go to question 12.*

2) **Are indicator shellfish species (also called 'sentinel' species) used to monitor marine biotoxins in the mixed production areas?**
   Yes: ☐
   No: ☐
   Please insert comments here:

*If you answered 'yes' to question 2, please answer questions 3 - 17. If you answered 'no', please go to question 12.*

3) **What species of shellfish is used as an indicator?**

4) **What species of shellfish are represented by the indicator?**

5) **Are scallops represented by an indicator species (i.e. is another species of shellfish used to indicate biotoxin uptake by scallops)?**
   Yes: ☐
   No: ☐
   Please insert comments here:

6) **Do you have concurrent monitoring data resulting from the analysis of both species (the indicator species and shellfish represented by the indicator species), or other validation data, to support the use of indicator species in the management programme?**
   Yes: ☐
   No: ☐
   Please insert comments here:

*If you answered 'yes' to question 6, please answer questions 7 - 17. If you answered 'no', please go to question 8.*

7) **Is the validation data published?**

Yes: ☐

No: ☐

Please provide references:

8) **What follow up actions are taken if toxins below regulatory level are detected in an indicator species? For example, is testing of other shellfish species (those that are represented by the sentinel) undertaken, or frequency of testing increased?**

9) **What follow up actions are taken if toxins above the regulatory limit are detected in the indicator species? For example, is harvesting of other shellfish species restricted (as well as for the indicator species)?**

10) **Once an area is closed due to toxicity above the regulatory level are shellfish indicator species used to indicate presence/absence of toxicity in other shellfish species in the same area to facilitate re-opening of a closed area (i.e. during the decontamination phase)?**

Yes: ☐

No: ☐

Please insert comments here:

11) **Have there been any instances in which an indicator species has tested negative (or been below the regulatory limit) and 'other' species of shellfish present in the same area have exceeded the regulatory limit for a particular toxin type?**

Yes: ☐

No: ☐

Please insert comments here:

12) **Are phytoplankton used as an indicator of shellfish toxicity in the management programme?**

Yes: ☐

No: ☐

Please insert comments here:

*If you answered 'yes' to question 12, please answer questions 13 to 17. If you answered 'no', please go to question 14.*

13) **Is plankton used as an indicator of toxicity for all regulated toxin groups, or for selected toxin groups (please specify which toxin groups phytoplankton counts are used to indicate toxicity in shellfish)?**

Paralytic shellfish toxins: ☐
Diarrhetic shellfish toxins: ☐
Azaspiracids: ☐
Amnesic shellfish toxins: ☐
Yessotoxins: ☐

Please insert comments here:

14) **Are any other technologies used as an indicator of toxicity in production areas e.g. the use of solid phase adsorption toxin tracking (SPATT) devices?**

Yes: ☐
No: ☐

If 'yes', please specify the type of technology that is used here, and the way in which the use of the technology informs management decisions:

15) **Do you use shellfish indicator species for monitoring environmental contaminants (e.g. heavy metals, PAH's, dioxins or PCB's)?**

Yes: ☐
No: ☐

*If you answered 'yes' to question 15, please answer question 16 and 17.*

16) **What species of shellfish is used as an indicator for chemical contaminants, and what species of shellfish does the indicator represent?**

17) **What chemical contaminants (e.g. heavy metals, PAH's, dioxins or PCB's) are monitored in the indicator species?**

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