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# Profiles of lipid soluble biotoxins in common mussels from Scottish production areas during the summer and autumn of 2006

Contract Reference: S14033

## Biotoxin Team Report

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**Final report**

**21st December 2006**

**18 pages**

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## 1. Introduction

Toxic metabolites produced by marine dinoflagellates (*Dinophysis*) can accumulate in filter-feeding shellfish and then be transmitted to the human consumer via the consumption of seafood. For the purposes of safeguarding public health, seafood products undergo regular monitoring for these toxins. The toxins associated with the human toxic syndrome – diarrhetic shellfish poisoning (DSP) can be divided into three groups based on their chemical structure; (i) the acidic *okadaic acid* (OA) and its derivatives *dinophysistoxins* (DTXs), neutral polyether lactones - *pectenotoxins* (PTXs), and sulphated polyethers - *yessotoxin* (YTX) and its disulphated analogues. Other lipid-soluble toxins such as spiroimines [e.g., azaspiracids (AZAs), gymnodimine (GYM) and spirolides (SPXs)] are also isolated during extraction. The customary method of monitoring DSP toxins is a lipophilic extraction followed by a live animal (mouse) bioassay (MBA) involving an intra-peritoneal (i.p.) injection and observations of the mouse survival time (1). The Lethal $Dose_{50}$ value for OA is ~200 µg/kg (i.p.) and concentrations less than this generally result in the mouse demonstrating symptoms of toxicity. The proposed regulatory limits are 160 µg/kg for OA, DTXs, PTXs and AZAs, and 1 000 µg/kg for YTXs (2).

Throughout the summer and autumn months of 2006, routine reports of MBA observations performed on common mussels (*Mytilus edulis*) from shellfish producing sites in Scotland indicated the appearance and wide diffusion of 'DSP' toxins. This led to (1) determining the concentrations in selected mussel samples and (2) exploring the toxin composition exhibited by these shellfish over a five-month period. The following describes the analytical approach, and presents qualitative, quantitative and confirmatory lipophilic toxin data.

## 2. Materials and methods

From 20th June to 18th October 2006, 30 common mussel samples demonstrating positive results attained by MBA, were selected for analysis of lipophilic toxins by liquid chromatography with mass spectrometric detection (LC-MS). Shellfish samples were acquired under the Official Control monitoring programme for Scotland from production sites located in the Shetland Isles, the Isles of Lewis and Harris, Skye and Mull, and from three sites situated in the north-western and western coastal areas in Scotland. The 'BTX/2006/' sample numbers are listed in the table of results and the corresponding 'FSA02-' sample numbers can be found in Appendix 1.

The sample set included mussels from Shetland (Vaila Sound, East of Linga, BTX/2006/917), which were classed as negative by the monitoring programme but led to some characteristic DSP clinical signs being displayed in the MBA, indicating the possibility of low-level toxicity. In addition, a further two samples from the Shetland Isles [BTX/2006/1547 (Busta Voe) and 1605 (Cribba Sound)] were also analysed as these had demonstrated 'atypical' responses during the live animal assays and a third sample (1636) was also included from Busta Voe, although no MBAs were performed on this sample.

Details of the grid references and latitude and longitude of the position of the sample locations are shown in Table 1, and Figure 1 geographically represents these positions. Aliquots of the whole mussel tissue homogenates that had been prepared for MBAs were archived and stored at –20 °C until LC-MS analyses.

A 2 g sub-sample of the homogenate was extracted with 80% methanol in deionised water. Alkaline hydrolysis (3) was undertaken on an aliquot of the methanolic extract to chemically-convert the low-polar 'DTX3' toxins (acyl esters of OA, DTX1 and/or DTX2) to the parent OA, DTX1and/or DTX2 toxins for direct LC-MS analyses. The crude, methanolic extract and the hydrolysed fraction were then analysed by LC-MS, and the amounts of 'DTX3s' were determined by the difference between the hydrolysed toxin contents and the 'free' or unhydrolysed quantities.

**Table 1: Geographical positions of mussel samples acquired under the Official Control monitoring for lipohilic biotoxins.**

| Shellfish harvesting area | Production site | Grid reference | Latitude Longitude |
|---|---|---|---|
| **SHETLAND** | | | |
| Busta Voe | Lee North | HU344649 | N60 22 01 W01 22 38 |
| Vaila Sound | East of Linga | HU242480 | N60 12 58 W01 33 51 |
| Clift Sound | Whal Wick | HU403363 | N60 06 35 W01 16 34 |
| Ronas Voe | Ronas Voe | HU310806 | N60 30 31 W01 26 11 |
| Cribba Sound | Vemetry Sound | HU302594 | N60 19 05 W01 27 15 |
| Gruting Voe | Seli Voe | HU281481 | N60 13 00 W01 29 38 |
| **ISLE OF LEWIS & HARRIS** | | | |
| Loch Roag | Torranish | NB154339 | N58 12 09 W06 50 40 |
| Loch Roag | Drovinish | NB136326 | N58 11 23 W06 52 24 |
| Loch Roag | Linngeam | NB133332 | N58 11 42 W06 52 45 |
| Loch Leurbost | Loch Leurbost | NB374247 | N58 08 02 W06 27 39 |
| **ISLE OF SKYE** | | | |
| Loch Slapin | Cruaidhlinn | NG562170 | N57 10 46 W06 02 01 |
| **ISLE OF MULL** | | | |
| Loch Scridain East | Loch Scridain | NM450250 | N56 20 57 W06 07 37 |
| **WEST COAST** | | | |
| Loch Laxford | Weavers Bay | NC211486 | N58 23 24 W05 03 36 |
| Little Loch | Broom | | |
| Loch Beag | Ardnambuth | NM728835 | N56 53 16 W05 43 46 |

To separate and then detect the lipid soluble toxins, an Agilent 1100 series liquid chromatograph (LC) coupled to a Waters/Micromass Quattro *micro* mass spectrometer (MS) was used. Initial mass spectral acquisition was carried out in selected ion recording (SIR) and in both positive and negative modes. For qualitative toxin screening, the protonated, deprotonated, ammonium or sodiated adduct ions of the toxins were monitored. Appendices 2 to 4 show the elution of the five groups of toxins and the mass to charge ratios (m/z) used during SIR acquisition. Multiple reaction monitoring (MRM) with one or two transitions in positive and negative modes then provided confirmation of positive identifications.

Quantitation was undertaken in SIR mode by comparing peak areas with calibrations of primary certified reference standards (*OA, PTX2, YTX, GYM, SPX1*), and with an authentic, non-certified AZA1 standard. Estimations of concentrations of the closely related analogues of these primary toxins [*i.e.*, DTX1, DTX2 (*OA*); PTX2 seco acid (*PTX2*); homo YTX, 45-OH YTX (*YTX*); AZA2, AZA3 (*AZA1*)] were determined by assuming equal molar responses to the primary toxins. The limits of quantitation (LOQ) for OA, PTX2 and AZA1 were in the range of 0.5 to 10 µg/kg.

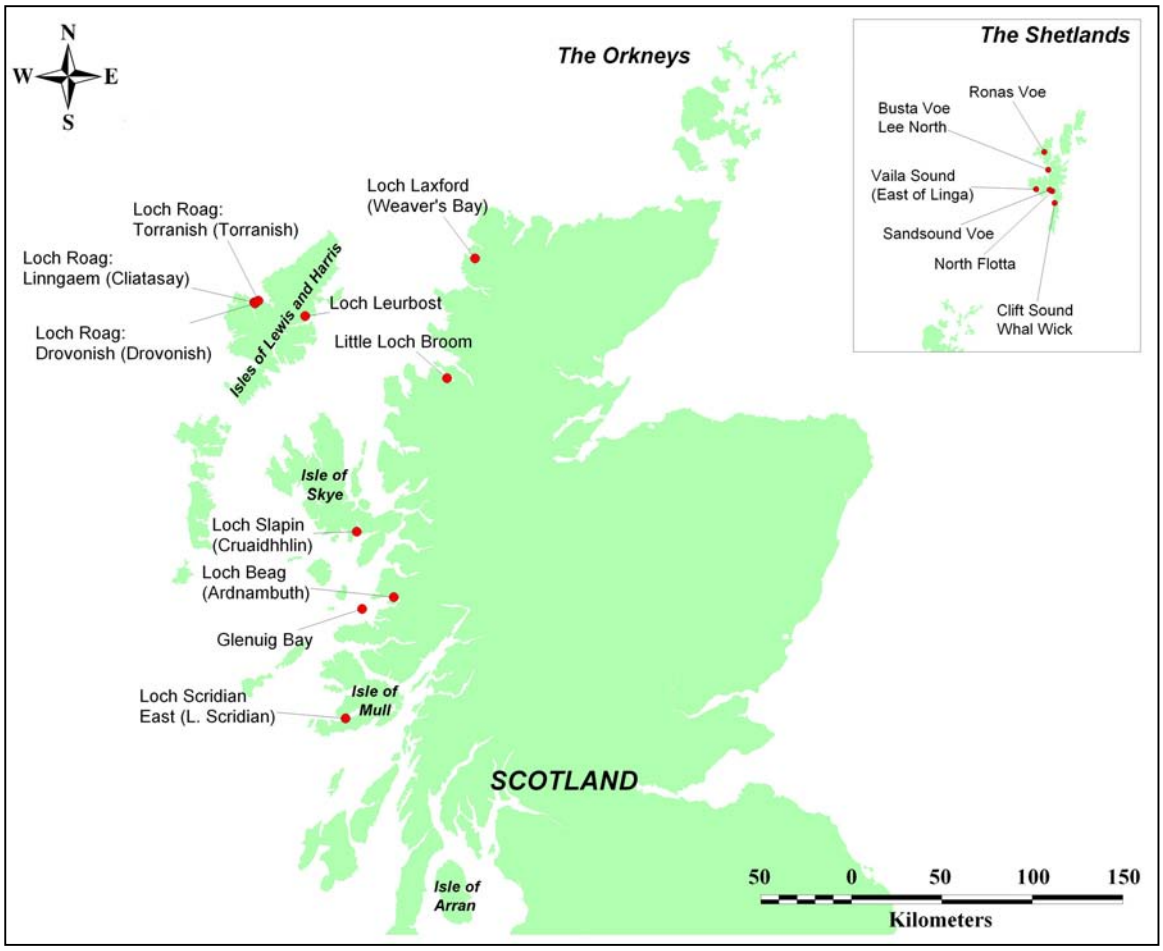



**Figure 1: Locations of common mussel samples acquired for the Official Control monitoring of lipophilic biotoxins in Scotland and for their determination by LC-MS.**

## 3. Results and Discussion

Concentrations (µg/kg) of lipid soluble toxins measured in common mussels and sampled between 20th June and 18th October 2006 are reported in Tables 2 to 5. None of the lipid soluble toxins measured in this study by LC-MS were detected in the negative MBA sample (BTX/2006/917) in which low-level toxicity had been suspected.

Analogues from four toxin groups (OA, PTX2, AZAs and spirolides) were detected throughout the study period whereas toxins belonging to the yessotoxin and gymnodimine groups were not detected in any of the samples analysed. The geographical spread of the samples permitted observations of the spatial distribution of the toxins, and analyses of samples acquired from the Shetland Isles and the Isles of Lewis and Harris enabled temporal trends of toxin profiles in these areas to be examined.

### 3.1 Spatial distributions of toxin groups and concentrations

#### *3.1.1. Okadaic acid and Dinophysistoxins*

This group not only showed the highest concentrations but the parent toxin, free OA was evident in all but one of the sample extracts analysed (Tables 2 and 3). The concentrations of free OA over all of the sites analysed ranged from 15 to 383 µg/kg. Levels >160 µg[OA]/kg (i.e. over the regulatory limit for this toxin) were detected in

Vaila Sound and Clift Sound (Shetland), at Drovinish and Linngeam (Loch Roag; Isle of Lewis and Harris) and at Loch Slapin (Isle of Skye).

Mass spectrometric (MRM) confirmatory data of OA, DTX1 and DTX2 toxins in selected samples are shown in Figure 2. For free DTX1 there were 13 positive LC-MS observations in shellfish samples, whereas free DTX 2 was detected in 18 samples. Only six samples showed evidence of both DTX1 and DTX2. In samples where both toxins analogues occurred together, higher concentrations of DTX1 were found in comparison to DTX2. The concentrations of DTX 1 detected in samples ranged from <LOQ to a maximum of 69 µg/kg (Linngeam, BTX/2006/923), while for DTX 2 concentrations ranged from <LOQ to 53 µg/kg (Clift Sound, Whal Wick, BTX/2006/1603).

Of the OA, DTX1 and DTX2 esters, those of OA predominated, followed by esters of DTX1 and then DTX2. In eight samples, levels of Σ(OA+DTX esters) were higher than the summation of free DSPs (i.e., ΣfreeOA + free DTXs). A maximum of 890 µg/kg of the total DSPs [ΣFree(OA+DTXs) + ΣEsters(OA+DTXs)] was detected at Clift Sound, Whal Wick (Shetland). In all samples where DSP toxins were detected, the contribution of Σ(free DSPs) and Σ(DSP esters) to total DSP concentrations ranged from 33 to 100 % and from 0 to 77 %, respectively.

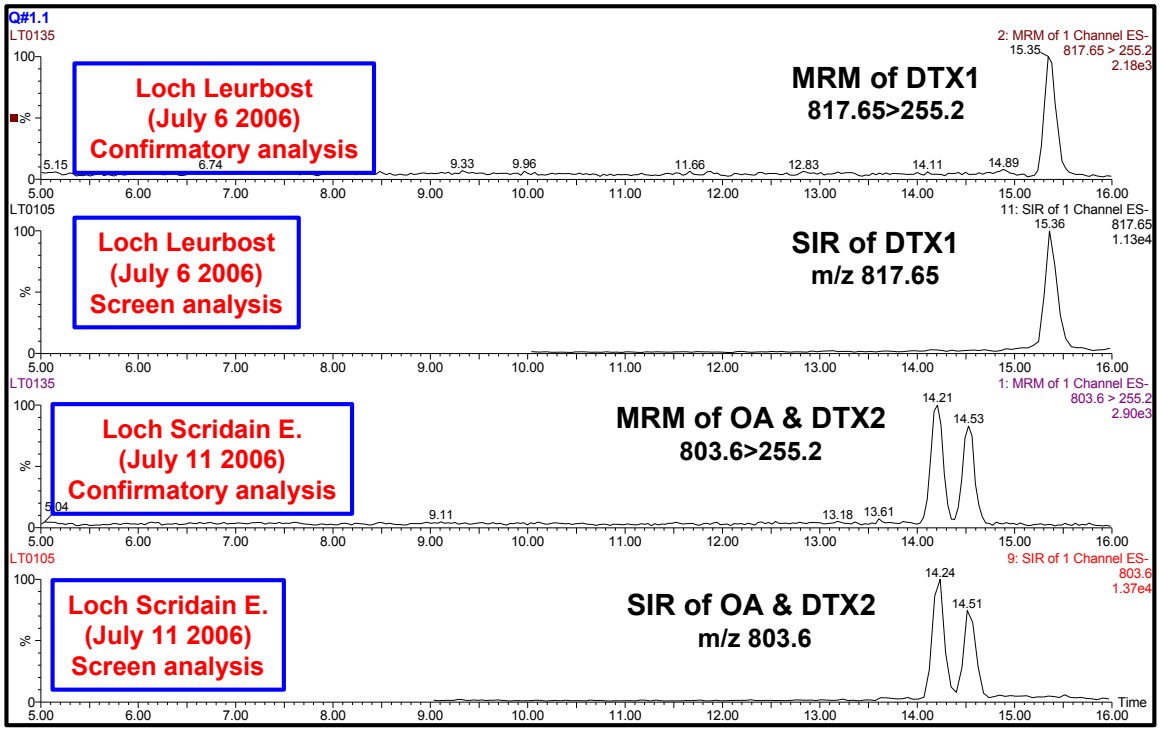



**Figure 2: Confirmatory MRM analysis of OA and DTX2 toxins in mussels from Loch Scridain East, and DTX1 from Loch Leurbost.**

**Table 2: Concentrations (µg/kg) of okadaic acid and dinophysistoxins in mussels acquired from the Shetland Isles (9th August to 18th October 2006).**

| Sample site location | BTX/2006 sample no. | Sample date (2006) | Toxin concentration (µg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Free OA | Free DTX1 | Free DTX2 | **ΣFree (OA+DTXs)** | OA esters | DTX1 esters | DTX2 esters | **ΣEsters (OA+DTXs)** | **Total DSPs (Free+Esters)** |
| **SHETLAND** | | | | | | | | | | | |
| ***Busta Voe*; Lee North** | 1333 | 09 Aug | 86 | ND | 20 | **106** | 34 | 9 | ND | **43** | **149** |
| | 1547 | 07 Sep | 50 | ND | ND | **50** | 44 | 7.4 | <LOQ | **51** | **101** |
| | 1636 | 13 Sep | 40 | ND | <LOQ | **40** | 46 | 6.8 | ND | **53** | **93** |
| ***Vaila Sound*; East of Linga** | 917 | 20 Jun | ND | ND | ND | **ND** | ND | ND | ND | **ND** | **ND** |
| | 1151 | 18 Jul | 66 | ND | ND | **66** | 95 | 37 | ND | **132** | **198** |
| | 1326 | 09 Aug | 229 | 24 | ND | **253** | 112 | 22 | ND | **134** | **387** |
| ***Clift Sound*; Whal Wick** | 1308 | 08 Aug | 365 | 53 | ND | **418** | 431 | 31 | ND | **462** | **890** |
| | 1489 | 30 Aug | 178 | 37 | 12 | **227** | 103 | 28 | ND | **131** | **346** |
| | 1530 | 05 Sep | 140 | 21 | 5.4 | **166** | 13 | 19 | ND | **32** | **198** |
| | 1603 | 12 Sep | 121 | 64 | 53 | **238** | 41 | ND | ND | **41** | **279** |
| ***Ronas Voe;* Ronas Voe** | 1330 | 09 Aug | 146 | 64 | 20 | **230** | 104 | ND | ND | **104** | **334** |
| | 1454 | 23 Aug | 75 | ND | ND | **75** | 93 | 14 | ND | **107** | **182** |
| | 1746 | 27 Sept | 73 | 54 | 22 | **149** | 32 | ND | ND | **32** | **181** |
| | 1796 | 04 Oct | 58 | ND | ND | **58** | 32 | ND | ND | **32** | **90** |
| | 1919 | 18 Oct | 53 | ND | 38 | **91** | 27 | ND | ND | **27** | **118** |
| ***Cribba Sound*** | 1605 | 12 Sep | 15 | ND | <LOQ | **15** | 25 | ND | ND | **25** | **40** |
| ***Gruting Voe;* Seli Voe** | 1690 | 20 Sep | 63 | ND | 6.9 | **70** | 24 | ND | ND | **24** | **94** |

**Table 3: Concentrations (µg/kg) of okadaic acid and dinophysistoxins in mussels acquired from the Isles of Lewis, Harris, Skye and Mull and from the west coast (21st June to 12th September 2006).**

| Sample site location | BTX/2006 sample no. | Sample date (2006) | Toxin concentration (µg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Free OA | Free DTX1 | Free DTX2 | **ΣFree (OA+DTXs)** | OA esters | DTX1 esters | DTX2 esters | **ΣEsters (OA+DTXs)** | **Total DSPs (Free+Esters)** |
| **ISLE OF LEWIS & HARRIS** | | | | | | | | | | | |
| *Loch Roag*; Torranish | 988 | 28 Jun | 136 | ND | ND | **136** | 67 | ND | ND | **67** | **203** |
| | 1609 | 12 Sep | 22 | ND | <LOQ | **22** | 13 | ND | ND | **13** | **35** |
| | 1264 | 02 Aug | 82 | ND | 5.3 | **87** | 64 | ND | 22 | **86** | **173** |
| *Loch Roag*; Drovinish | 928 | 21 Jun | 357 | 60 | ND | **417** | 28 | ND | ND | **28** | **445** |
| | 986 | 28 Jun | 182 | ND | ND | **182** | 34 | 15 | ND | **49** | **231** |
| | 1167 | 19 Jul | 119 | 38 | ND | **157** | 129 | 38 | ND | **167** | **324** |
| | 1251 | 02 Aug | 154 | 12 | <LOQ | **166** | 179 | 81 | ND | **260** | **426** |
| *Loch Roag*; Linngeam | 923 | 21 Jun | 383 | 69 | ND | **452** | ND | ND | ND | **ND** | **452** |
| *Loch Leurbost* | 1046 | 06 Jul | 143 | 23 | ND | **166** | 154 | 21 | ND | **175** | **341** |
| | 1169 | 19 Jul | 111 | ND | ND | **111** | 97 | ND | ND | **97** | **208** |
| | 1205 | 25 Jul | 200 | ND | 45 | **245** | 73 | ND | ND | **73** | **318** |
| **ISLE OF SKYE** | | | | | | | | | | | |
| *Loch Slapin* | 971 | 27 Jun | 219 | 36 | ND | **255** | 38 | ND | ND | **38** | **293** |
| **ISLE OF MULL** | | | | | | | | | | | |
| *Loch Scriddian East* | 1098 | 11 Jul | 84 | ND | 13 | **97** | 69 | 5 | 7 | **81** | **178** |
| **WEST COAST** | | | | | | | | | | | |
| *Loch Laxford* | 1099 | 11 Jul | 63 | ND | 13 | **76** | 59 | ND | 5 | **64** | **140** |
| *Little Loch Broom* | 1381 | 16 Aug | 17 | ND | 2.8 | **20** | 13 | ND | ND | **13** | **33** |
| *Loch Beag* | 1212 | 25 Jul | 103 | ND | 17 | **120** | 84 | ND | 6 | **90** | **210** |

### *3.1.2 Pectenotoxin 2 and its metabolite*

Concentration data for this group of lipophilic toxins can be found in Tables 4 and 5. Levels of the parent PTX2 toxin ranged from <LOQ to 418 µg/kg (Drovinish, Loch Roag, Isle of Harris). Samples from Torranish and Drovinish (Loch Roag, Isle of Harris), and from Loch Slapin (Cruaidhlinn, Isle of Skye) exhibited levels >160 µg[PTX2]/kg. Figure 3 shows LC-MS/MS MRM confirmation of the detection of PTX2 in the Loch Drovinish sample (BTX/2006/986).

Whereas the OA group dominated in mussels from Shetland, PTX2 was only evident in these samples at low concentrations (<5 µg/kg). In all samples in which PTX2 was detected, it was accompanied by its hydrolysis metabolite – PTX seco acid (PTX2sa). PTX2sa was apparent in 79 % of the samples [<LOQ to 120 µg/kg (Loch Laxford; Weavers Bay)]. PTX2 was absent in 23 % of these samples. However, when used a toxin marker, the presence PTX2sa in those samples that showed no PTX2 contamination is indicative that, at some point in time, the mussels had possibly accumulated the parent toxin.

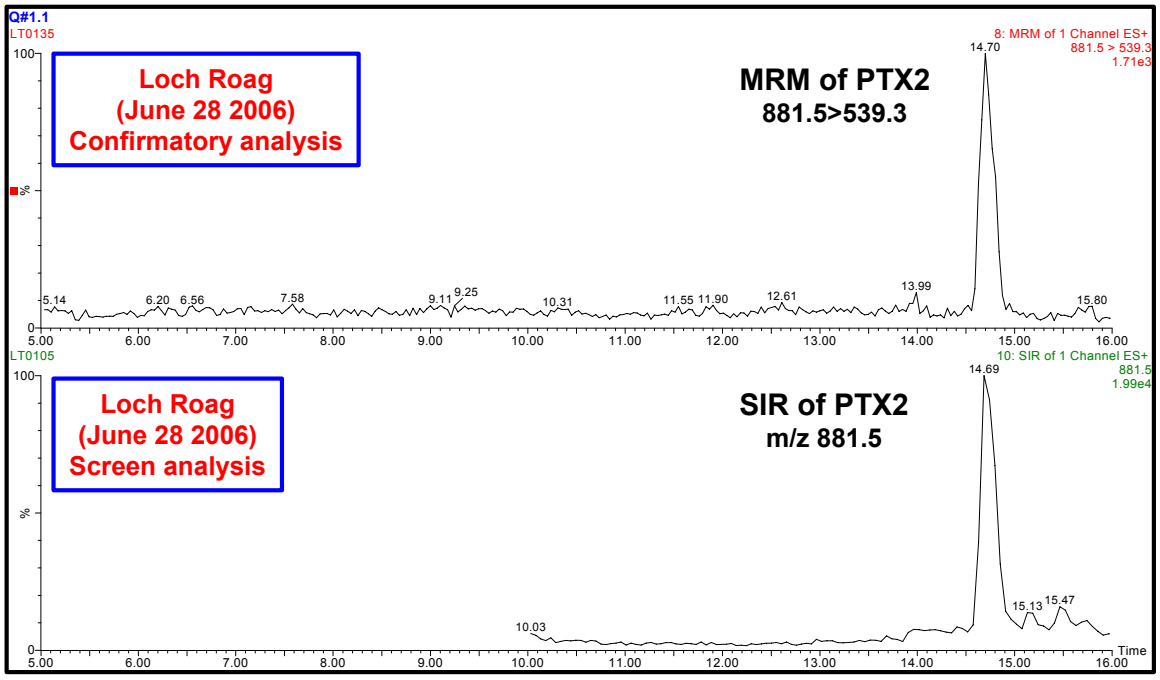



**Figure 3: MRM confirmations of PTX2 SIR observations in mussels from L. Drovinish, L. Roag (28 June 2006).**

### *3.1.3. Azaspiracid toxins*

Analogues of AZA were detected during the course of this study, although the distribution of these toxins was less widespread in comparison to the OA or PTX groups (Tables 4 and 5). Overall, concentrations were found to be <10 µg/kg, with AZA analogues detected in a total of 12 samples. Of the three AZA analogues, AZA1 predominated. AZA1 was detected in all 12 samples, while AZA 2 was detected in seven samples and AZA 3 in two samples. Both AZA 2 and AZA 3 were found only in samples where AZA1 was also detected. Where AZA1 only was observed, it is possible that AZA2 and/or 3 may also have been present but at levels <LOD of the methodology.

AZAs were only identified in localised areas of Shetland including Ronas Voe, Busta Voe and Cribba Sound as well being as detected at all of the Loch Roag shellfish production sites. Confirmation by LC-MS/MS of the detection of AZA1 in the Loch Leurbost sample BTX/20061046 is shown in Figure 4. Interestingly, AZA toxins were also detected in cockles and mussels from the Camel estuary (Devon, England) during September 2006. The detection of AZAs in Scottish shellfish occurred earlier in the summer and concentrations were approximately three times lower than those found in Camel shellfish.

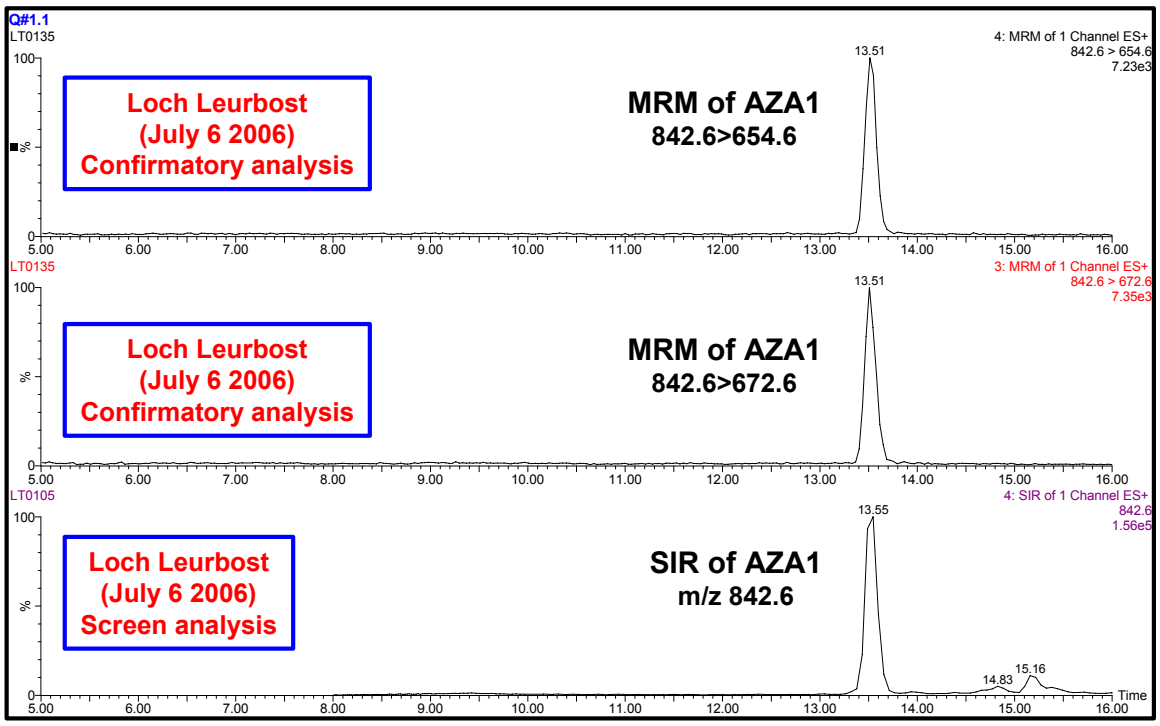



**Figure 4: Confirmation of AZA1 in mussels from Loch Leurbost by MRM experiments and using two transitions ions [842.6>672.6 and 842.6>654.6].**

### *3.1.4 Desmethyl spirolide C*

The parent (desmethyl spirolide C; SPX1) toxin represented this group and was evident at three locations (Loch Roag, Loch Scridain East and Loch Beag; Table 5). The quantities of this toxin were measured at trace (<1.5 µg/kg) levels and its presence may have been a product of a long-term residue from a previous *Alexandrium ostenfeldii* bloom event.

**Table 4: Concentrations of pectenotoxin 2s, azaspiracids, yessotoxins, desmethyl spirolide C (SPX1) and gymnodinium toxins in common mussels acquired from Shetland (20th June to 18th October 2006).**

| Sample site location | BTX/2006 sample no. | Sample date (2006) | Toxin concentration (µg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PTX2 | PTX2sa | AZA1 | AZA2 | AZA3 | YTX | homo YTX | SPX1 | GYM |
| **SHETLAND** | | | | | | | | | | | |
| ***Busta Voe*; Lee North** | 1333 | 09 Aug | <LOQ | 0.57 | ND | ND | ND | ND | ND | ND | ND |
| | 1547 | 07 Sep | ND | <LOQ | 8.6 | 4.9 | ND | ND | ND | ND | ND |
| | 1636 | 13 Sep | ND | <LOQ | 7.8 | 6.1 | ND | ND | ND | ND | ND |
| ***Vaila Sound*; East of Linga** | 917 | 20 Jun | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| | 1151 | 18 Jul | 5.0 | 37 | ND | ND | ND | ND | ND | ND | ND |
| | 1326 | 09 Aug | ND | <LOQ | ND | ND | ND | ND | ND | ND | ND |
| ***Clift Sound*; Whal Wick** | 1308 | 08 Aug | <LOQ | 4.1 | ND | ND | ND | ND | ND | ND | ND |
| | 1489 | 30 Aug | 3.9 | 6 | ND | ND | ND | ND | ND | ND | ND |
| | 1530 | 05 Sep | ND | 4.0 | ND | ND | ND | ND | ND | ND | ND |
| | 1603 | 12 Sep | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| ***Ronas Voe*; Ronas Voe** | 1330 | 09 Aug | ND | 6 | ND | ND | ND | ND | ND | ND | ND |
| | 1454 | 23 Aug | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| | 1746 | 27 Sep | ND | ND | 3.6 | 1.2 | ND | ND | ND | ND | ND |
| | 1796 | 04 Oct | ND | ND | 3.1 | 0.29 | ND | ND | ND | ND | ND |
| | 1919 | 18 Oct | ND | ND | 4.0 | ND | ND | ND | ND | ND | ND |
| ***Cribba Sound*** | 1605 | 12 Sep | ND | 0.73 | 3.9 | ND | ND | ND | ND | ND | ND |
| ***Gruting Voe*; Seli Voe** | 1690 | 20 Sep | ND | ND | ND | ND | ND | ND | ND | ND | ND |

**Table 5: Concentrations of pectenotoxin 2s, azaspiracids, yessotoxins, desmethyl spirolide C (SPX1) and gymnodinium toxins in common mussels acquired from the Isles of Lewis, Harris, Skye and Mull and from the west coast (21th June to 12th September 2006).**

| Sample site location | BTX/2006 sample no. | Sample date (2006) | Toxin concentration (µg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PTX2 | PTX2sa | AZA1 | AZA2 | AZA3 | YTX | homo YTX | SPX1 | GYM |
| **ISLES OF LEWIS & HARRIS** | | | | | | | | | | | |
| *Loch Roag*; Torranish | 988 | 28 Jun | 267 | 49 | ND | ND | ND | ND | ND | ND | ND |
| | 1609 | 12 Sep | 4.0 | 5.5 | 6.6 | 2.2 | ND | ND | ND | ND | ND |
| | 1264 | 02 Aug | 2.3 | 14 | 5.0 | 1.7 | 1.5 | ND | ND | ND | ND |
| *Loch Roag*; Drovinish | 928 | 21 Jun | 25 | 97 | 4.7 | ND | ND | ND | ND | ND | ND |
| | 986 | 28 Jun | 418 | 37 | ND | ND | ND | ND | ND | ND | ND |
| | 1167 | 19 Jul | 3.5 | 23 | ND | ND | ND | ND | ND | ND | ND |
| | 1251 | 02 Aug | <LOQ | 7.6 | 7.0 | ND | ND | ND | ND | ND | ND |
| *Loch Roag*: Linngeam | 923 | 21 Jun | 21 | 90 | 4.7 | ND | ND | ND | ND | ND | ND |
| *Loch Leurbost* | 1046 | 06 Jul | 5.9 | 50 | 4.5 | <LOQ | <LOQ | ND | ND | 1.4 | ND |
| | 1169 | 19 Jul | 3.8 | 30 | ND | ND | ND | ND | ND | ND | ND |
| | 1205 | 25 Jul | 5.3 | 54 | ND | ND | ND | ND | ND | ND | ND |
| **ISLE OF SKYE** | | | | | | | | | | | |
| *Loch Slapin* | 971 | 27 Jun | 365 | 22 | ND | ND | ND | ND | ND | ND | ND |
| **ISLE OF MULL** | | | | | | | | | | | |
| *Loch Scriddian East* | 1098 | 11 Jul | 6.4 | 77 | ND | ND | ND | ND | ND | 1.4 | ND |
| **WEST COAST** | | | | | | | | | | | |
| *Loch Laxford* | 1099 | 11 Jul | 17 | 120 | ND | ND | ND | ND | ND | ND | ND |
| *Little Loch Broom* | 1381 | 16 Aug | >LOQ | 6.2 | ND | ND | ND | ND | ND | ND | ND |
| *Loch Beag* | 1212 | 25 Jul | 3.2 | 33 | ND | ND | ND | ND | ND | 0.35 | ND |

## 3.2 Temporal trends of toxin concentrations

At three shellfish production sites (Drovinish in Loch Roag, and Clift Sound and Ronas Voe in Shetland), toxin concentrations were examined over the 6-month period of the study to identify possible temporal trends over discrete sampling periods. Although the frequency of sampling was not highly resolved, general trends in toxin profile and levels of contamination detected in these sites have been presented for the sampling periods studied.

### *3.2.1 Lipophilic toxin trends in mussels from Drovinish, L. Roag*

Four samples from Drovinish were analysed between 21st June and 2nd August 2006 and a profile of the dominant toxins is presented in Figure 5.

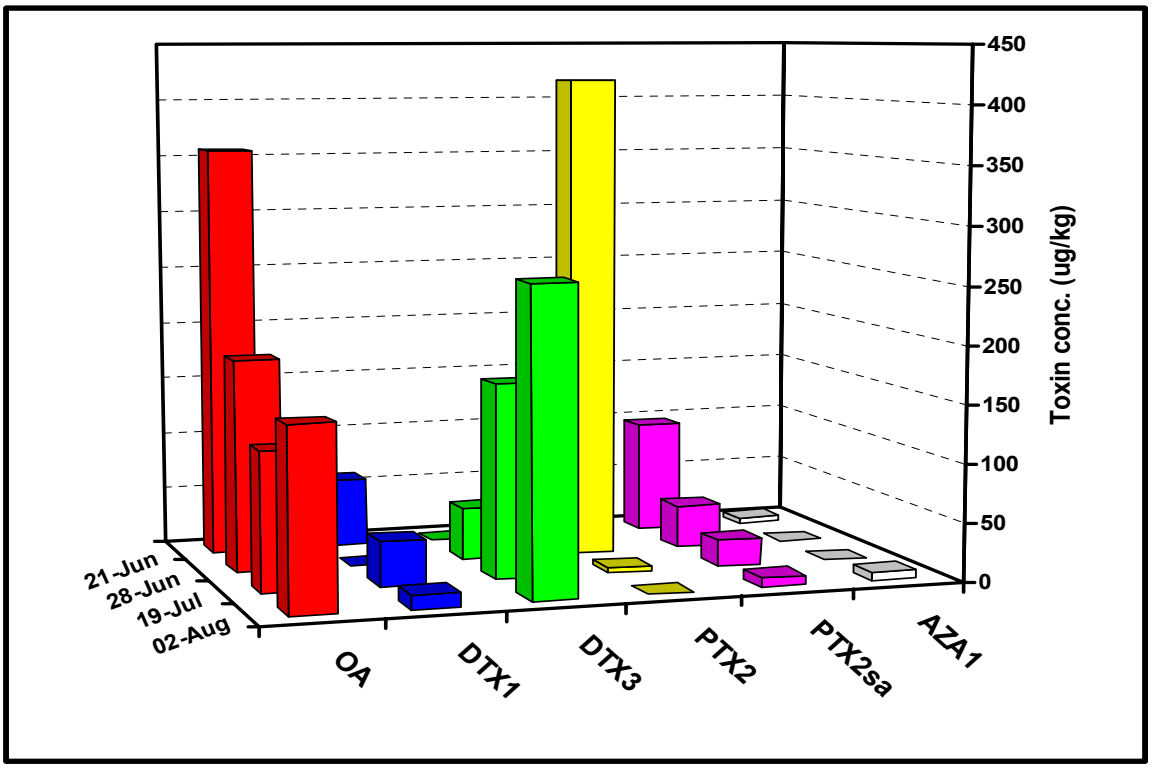



**Figure 5: Temporal trends of OA/DTXs, PTX2/2sa and AZA1 toxins in mussels from Drovinish, L. Roag (21st June to 02nd August 2006).**

Maximum OA and PTX2 levels occurred within the first two samples (21st June and 28th June), with PTX2 levels exceeding 400 µg/kg in the first sample. OA values appeared to decrease by 66 % over the four-week period to 19 July. However, PTX2 declined rapidly to ~1 % of the maxima within the same time period. Levels of the total (OA+DTXs) esters or 'DTX3s' in shellfish from this location demonstrated a reverse trend to free OA/DTX1 and increased over the seven-week period from Not Detected to 260 µg/kg. A step-wise decrease in the PTX2 metabolite (PTX2sa) was apparent, however there was no obvious or expected elevation in PTX2sa following the parent toxin maxima. The slight elevation in OA concentrations from 19th July to 2nd August may indicate an *intrasite* variability of contamination or a reoccurrence of a dinoflagellate episode in this locality and throughout this period. AZA1 was also evident, albeit at low levels, in mussels from this area and from mid-June and early

August. The absence of AZA in shellfish sampled in late June and July may be again, a product of *intrasite* variability.

### *3.2.2. Temporal trends of OA toxins in mussels from Clift Sound, Shetland*

For the six-week period between 8th August and 12th September, trends in concentrations of OA, DTX1, DTX2 and DTX3 (summation of OA and DTX esters) in mussels from Clift Sound are represented in Figure 6.

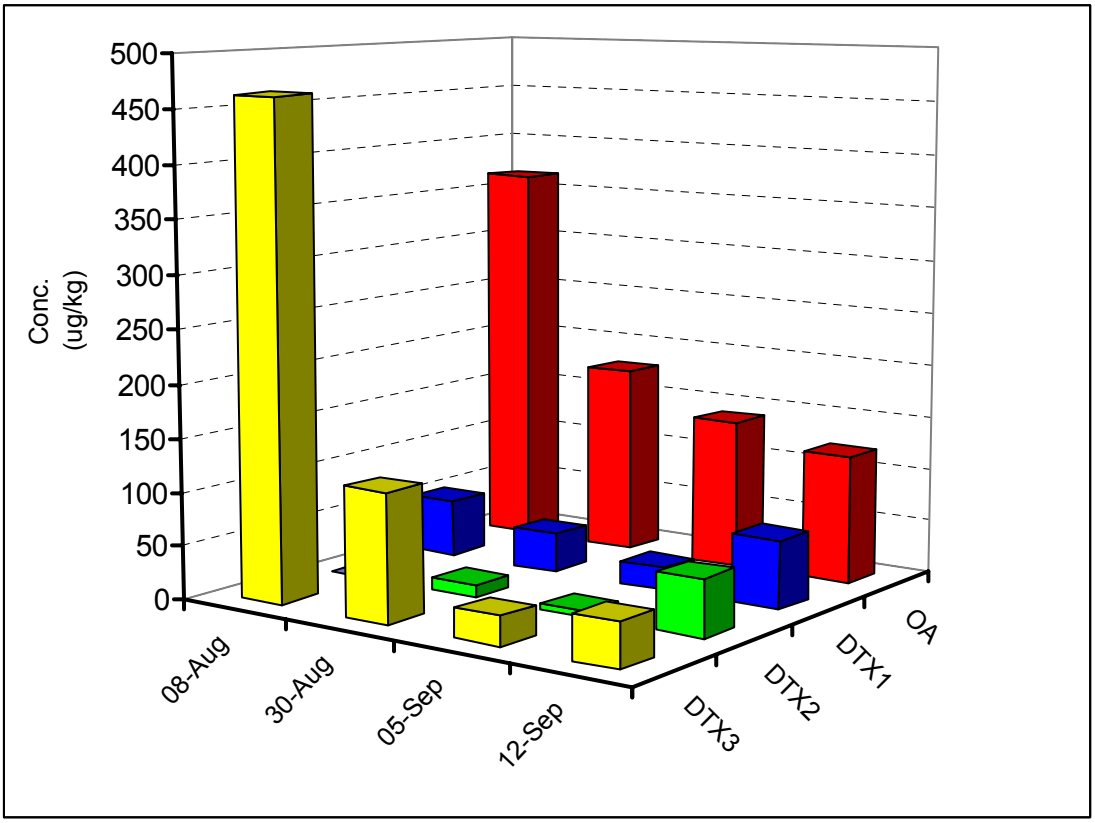



**Figure 6: Concentrations of OA and DTX toxins in mussels from Clift Sound, Shetland (8th August to 12th September 2006).**

Levels of OA decreased by a step-wise function and an approximate 50 % reduction in concentrations was seen to occur over 22 days (8th to 30th August). DTX1 also declined in concentration between 8th August and 5th September, but then increased approximately three-fold to a level >60 µg/kg over a seven day period. Over the same seven-day period, a ten-fold increase in DTX2 concentration was also observed, which was possibly a product of an emerging dinoflagellate bloom in the vicinity of the production site. A steep decline of >70 % in DTX3 concentrations was seen over the 23 day period between the first and second samples, and total esters were composed of those of OA and DTX1; there was no evidence of DTX2 esters in these samples.

### *3.2.3. Temporal trends of OA toxins in mussels from Ronas Voe, Shetland*

Between 9th August and 18th October 2006, five mussel samples were acquired from Ronas Voe, Shetland. Concentrations of the OA and DTX toxins are presented in Figure 7.

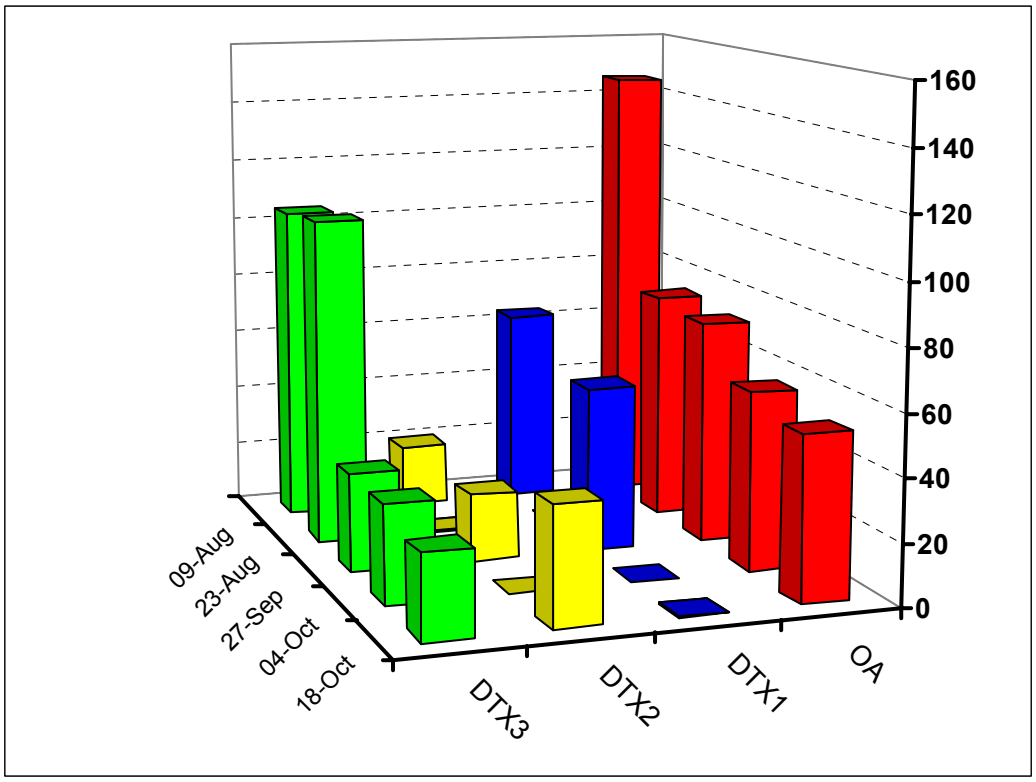



**Figure 7: Temporal trends of OA, DTX1, DTX2 and total OA+DTX esters (DTX3) toxins in mussels from Ronas Voe, Shetland (9th August to 18th October 2006).**

Okadaic acid concentrations were observed to decrease by ~50 % over the 15 day period between 9th and 23rd August. A small decrease of 15 µg[OA]/kg was observed after this date. DTX1 and DTX2 toxins were intermittent in their presence and there was no evidence of the former after September. The reoccurrence of DTX2 throughout the sampling period again may have resulted from newly emerging dinoflagellate blooms in the area. A sharp decline (~70 %) in DTX3 (OA esters + DTX esters) levels was observed after the 23rd August, and concentrations of ~ 30 µg[DTX3] /kg were then seen throughout late September and mid-October. Esters of OA contributed to DTX3 concentrations although DTX1 esters were apparent in mussels sampled on 23rd August.

## 4. Conclusions

- Comprehensive LC-MS analyses has proved to be a robust and a powerful tool to describe the spatial and temporal distributions of lipid-soluble toxin profiles in mussels from selected Scottish shellfish production sites during 2006.
- Some of these toxins were detected over a wide geographical area and temporal trends of okadaic acid and dinophysistoxins, at selected locations, indicated their presence was persistent for up to several months
- The OA group was frequently detected and intoxication of mussels with other toxin groups was apparent and short-lived as in the case of PTX2
- The co-occurrence of OA- and DTX-producing *Dinophysis* species were the likely source of the complex OA/DTX/PTX2 profiles in the shellfish
- It is important to include the chemical conversion of the 'DTX3s' during routine monitoring as these represent a potential toxic fraction.

## 5. References

(1) Yassumoto, T., Oshima, Y., Yamaguchi, M. (1979). In: *Toxic Dinoflagellates Blooms*. D, Taylor, H.H. Seliger (Eds). Amsterdam, The Netherlands, pp. 495-502.

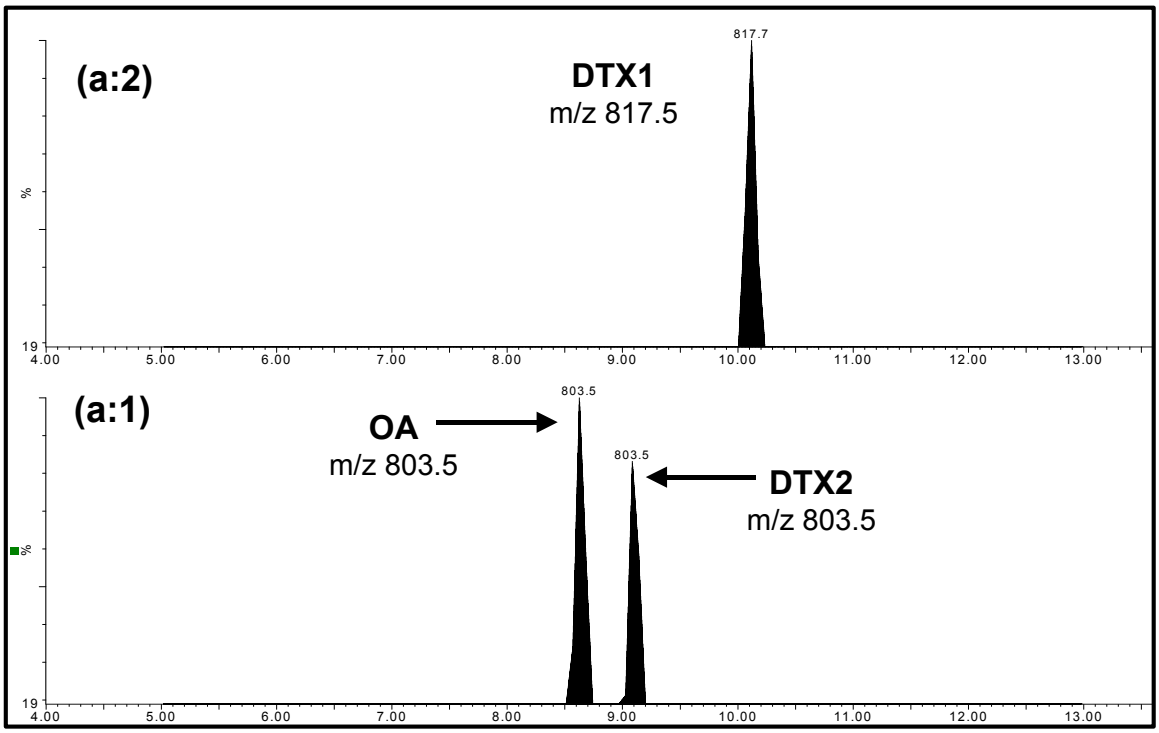
(2) European Commission Decision 2002/225/EC (2002). *Off. J. Eur. Commun*. **L175**, 62-64.

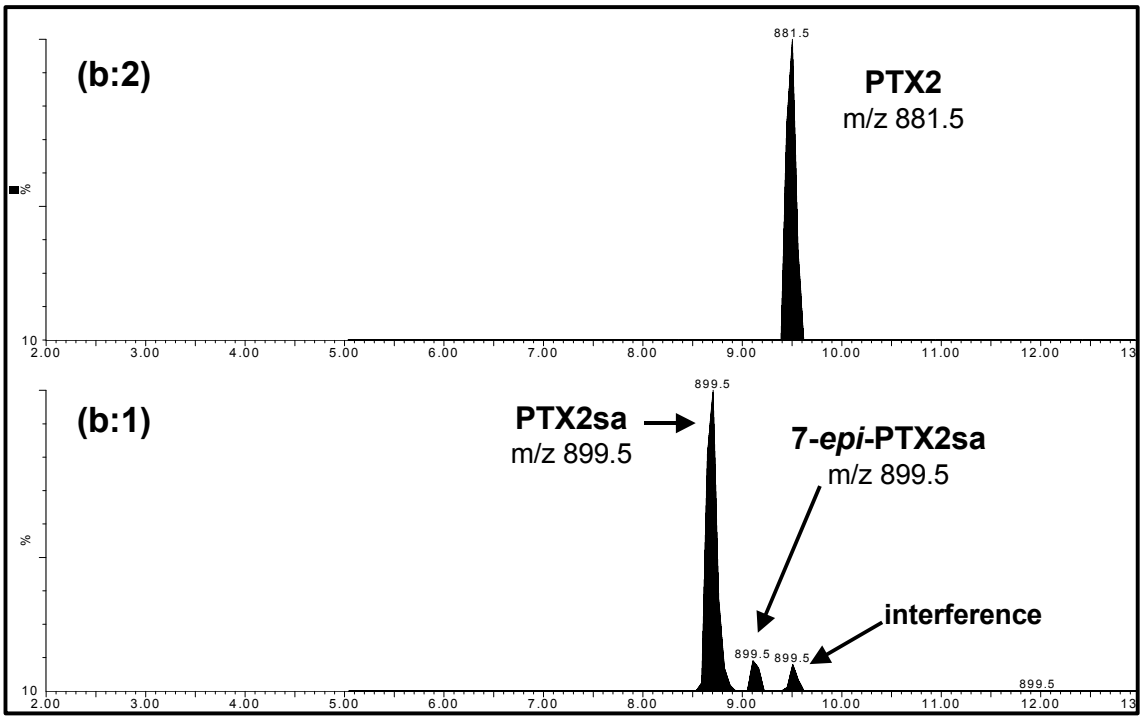
(3) Mountford, D.O., Suzuki, T., Truman, P. (2001). *Toxicon*, 39, 383-390.

**Appendix 1**. Assigned 'BTX/2006/' sample numbers and corresponding 'FSA02-' numbers.

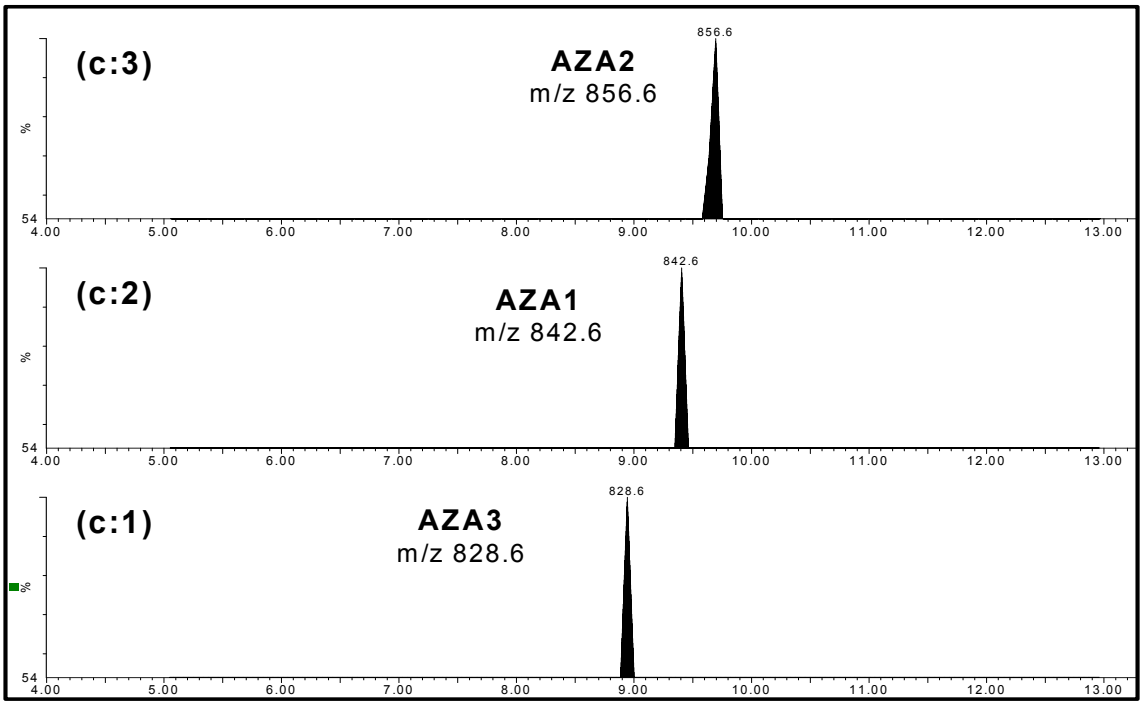
| BTX/2006/ sample no. | FSA02- sample no. | BTX/2006/ sample no. | FSA02- sample no. |
|---|---|---|---|
| 917 | 200606-03W | 1326 | 090806-14W |
| 923 | 210606-01W | 1330 | 090806-18W |
| 928 | 210606-06W | 1333 | 090806-21W |
| 971 | 270606-05W | 1381 | 160806-09W |
| 986 | 280606-05W | 1454 | 230806-07W |
| 988 | 280606-07W | 1489 | 300806-18W |
| 1046 | 060706-02W | 1530 | 050906-13W |
| 1098 | 110706-04W | 1547 | 070906-03W |
| 1099 | 110706-05W | 1603 | 120906-05W |
| 1151 | 180706-01W | 1605 | 120906-07W |
| 1167 | 190706-07W | 1609 | 120906-11W |
| 1169 | 190706-09W | 1636 | 130906-14W |
| 1205 | 250706-04W | 1690 | 200906-14W |
| 1212 | 250706-11W | 1746 | 270906-08W |
| 1251 | 020806-02W | 1796 | 041006-03W |
| 1264 | 020806-15W | 1919 | 181006-02W |
| 1308 | 080806-04W | | |

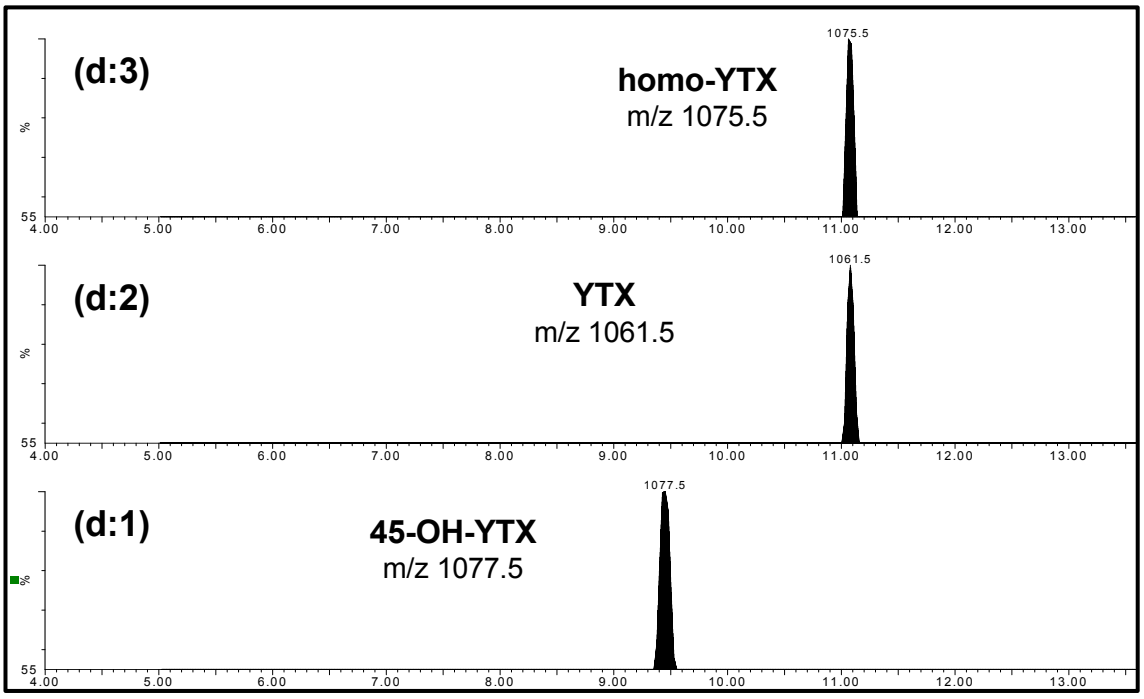
**Appendix 2.** Selected Ion Recording chromatograms of: (a:1) OA and DTX2, and (a:2) DTX1; and (b:1) PTX2sa and (b:2) PTX2.



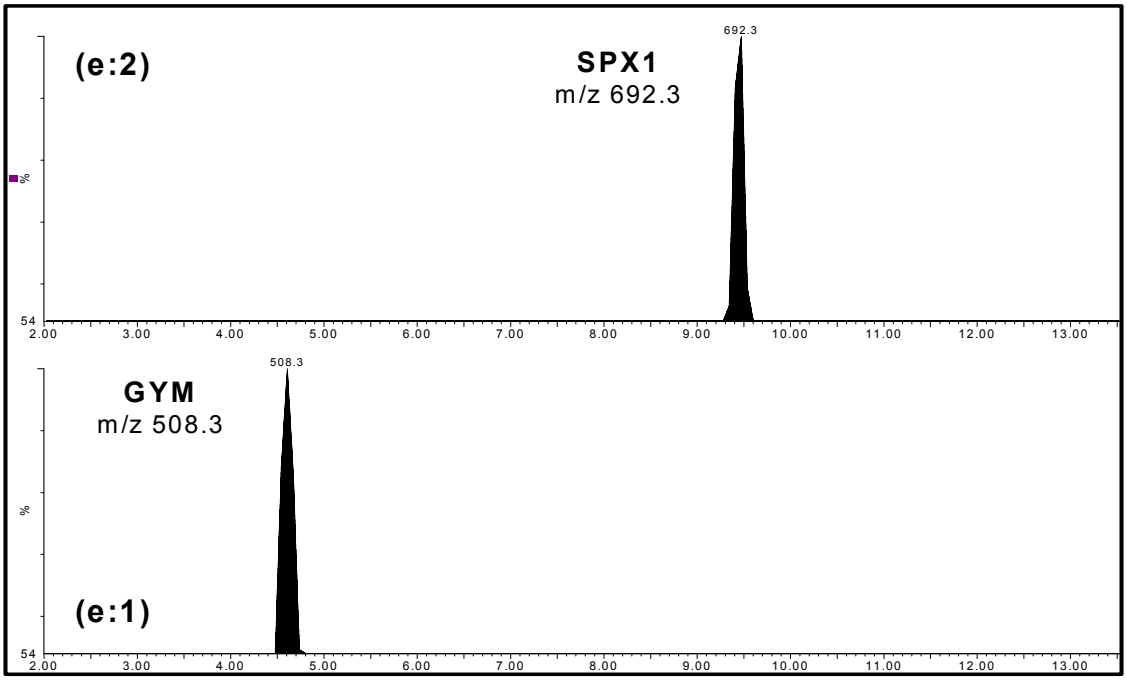



**Appendix 3.** Selected Ion Recording chromatograms of (c:1) AZA3, (c:2) AZA1 and (c:3) AZA2; and (d:1) 45-OH YTX, (d:2) YTX and (d:3) homo YTX.



**Appendix 4.** Selected Ion Recording chromatograms of (e:1) GYM and (e:2) SPX1.



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