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Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for Scotland - 2016

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Authors: Lewis Coates [(1)], Sarah Swan [(2)], Keith Davidson [(2)], Andrew Turner [(1)], Ben Maskrey [(1)] and Myriam Algoet [(1)]

1) Cefas Laboratory, Barrack Road, Weymouth, Dorset, DT4 8UB
2) The Scottish Association for Marine Science (SAMS), Scottish Marine Institute, Oban, Argyll, PA37 1QA

Document prepared by:	Lewis Coates, Cefas & Sarah Swan, SAMS - SRSL	
Document checked by:	Myriam Algoet, 16/02/17	Review Date: N/A
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Quality statement: This report is a compilation of the information included on the reports provided daily/weekly to FSS and showing the results of the phytoplankton and toxin analyses undertaken on samples submitted via the Official Control programme. All results were quality checked and approved prior to release to FSS and the results compiled in this report have been further checked against a copy of the original reports held on a central database. Information relating to the origin of the samples (place (including co-ordinates), date and time of collection) is as provided by contracted sampling staff and has not undergone verification checks by Cefas.

 The co-ordinates used to depict the location of the monitoring points are the default co-ordinates of the RMP or AHA sampling points defined by FSS.

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1. Summary

This report describes the results of the Official Control Biotoxin and Phytoplankton Monitoring Programmes for Scotland for the period 1st January to 31st December 2016.

The laboratory analyses for biotoxins in shellfish, co-ordination of the programme and its logistics were conducted by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) Weymouth Laboratory, whilst the laboratory phytoplankton analyses, co-ordination of the programme and its logistics were performed by the Scottish Association for Marine Science (SAMS - SRSL) in Oban, under the scope of the contracted Shellfish Partnership.

The programmes were delivered on behalf of Food Standards Scotland (FSS), the national competent authority for food safety and are aimed at delivering the testing required for the statutory monitoring of biotoxins in shellfish and for identification and enumeration of potentially harmful algal species in selected shellfish harvesting areas, as described in EC Regulations 854/2004, 882/2004 and 2074/2005.

Toxin monitoring

A total of 2,882 bivalve shellfish samples from 83 inshore sampling locations (Figure 1) were submitted to Cefas for toxin analyses in the reporting period. They comprised of: common mussels (1,875), Pacific oysters (543), razors (254), common cockles (138), surf clams (42), carpet clams (16) and Queen Scallops (14).

Twenty-eight king scallop samples were also collected from 12 commercial establishments under the scope of the FSS official control verification programme and were submitted for toxin analysis during the reporting period.

Thirteen inshore samples (<0.5% of those received) were rejected on arrival at the laboratory – five of these were submitted in error as testing was not required in these areas, another five arrived at the laboratory in a condition unsuitable for analyses, two samples arrived after the Christmas closure deadline and one sample was supplied without paperwork.

All samples received and assessed as suitable for testing provided sufficient material to perform all the required analyses.

Phytoplankton monitoring

A total of 1,305 seawater samples from 53 inshore sampling locations (Figure 2) were submitted to SAMS Research Services Ltd. (SRSL) for the identification and enumeration of potentially harmful algal species during the reporting period and 1,301 were analysed. Four samples were not analysed as they were not required, either due to the reduced autumn sampling schedule (three samples), or because the collection site was not on the current phytoplankton RMP list (one sample).

Table 1: Maximum Permitted Limits of toxins in shellfish flesh

Toxin group	Maximum Permitted Limits
ASP	>20 mg Domoic/epi-domoic acid/kg [shellfish flesh]
LTs	Diarrhetic shellfish poisoning (DSP) toxins and pectenotoxins (PTXs) together, >160µg okadaic acid eq./kg [shellfish flesh] or Yessotoxins, >3.75mg yessotoxin eq./kg [shellfish flesh] or Azaspiracids, >160µg azaspiracid eq./kg [shellfish flesh]
PSP	>800µg saxitoxin eq./kg [shellfish flesh]

For biotoxin and phytoplankton monitoring results for individual RMPs (Representative Monitoring Points) please visit the Scotland's Aquaculture website at the following links. All results are compared to the maximum permitted levels (Table 1) as stipulated in EC regulation 853/2004 (Section VII, Chapter V: Health standards for live bivalve molluscs):

Biotoxin monitoring -
http://aquaculture.scotland.gov.uk/data/biotoxin_monitoring_sample.aspx

Phytoplankton monitoring –
http://aquaculture.scotland.gov.uk/data/phytoplankton_monitoring_samples.aspx

Figure 1: Scottish inshore shellfish sampling locations – Food Standards Scotland biotoxin monitoring programme in 2016

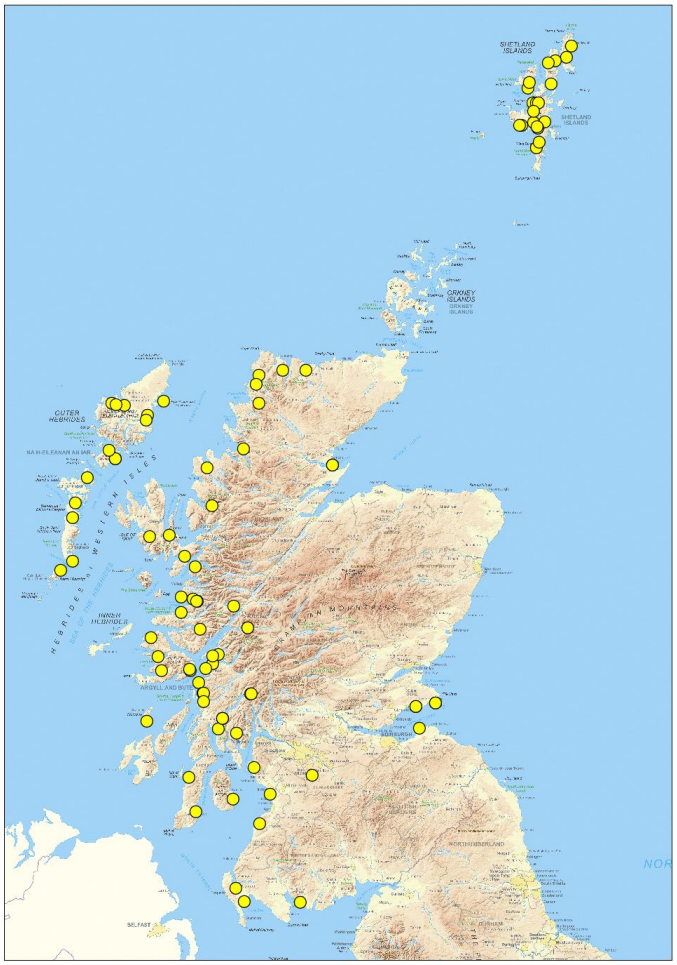
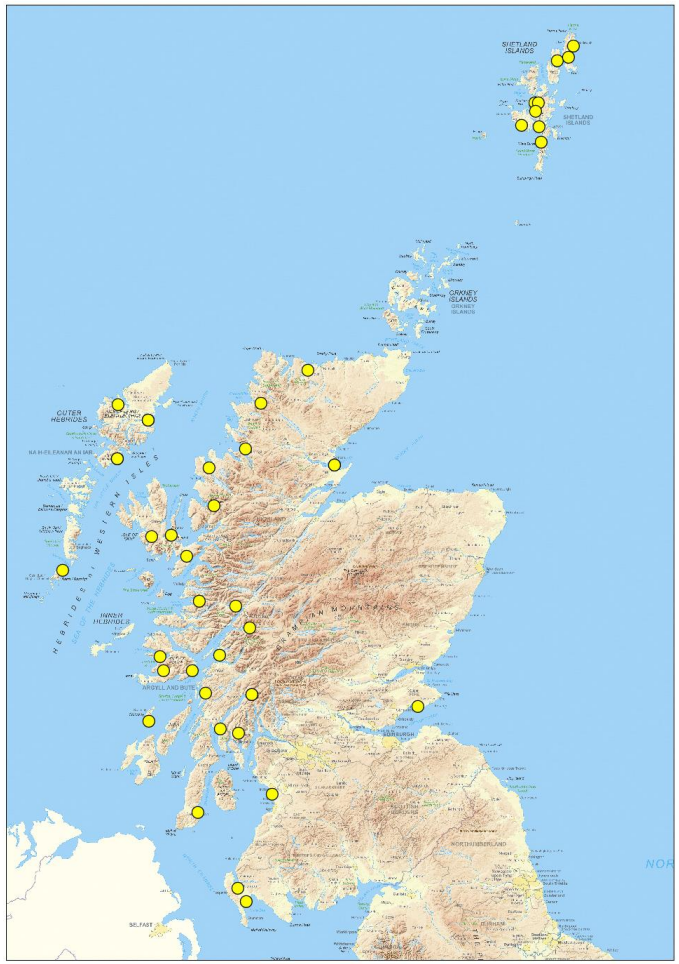


Figure 2: Scottish water sampling locations – Food Standards Scotland phytoplankton monitoring programme in 2016



Monitoring for lipophilic toxins

Monitoring for lipophilic toxins (LTs) was conducted using a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. The method is able to characterise and quantify the following LT groups; Okadaic Acid (OA)/Dinophysis Toxins (DTXs) and Pectenotoxins (PTXs) – reported as µg OA equivalent (eq.)/kg shellfish flesh, Azaspiracid toxins (AZAs) – reported as µg AZA1 eq./kg shellfish flesh and Yessotoxins (YTXs) reported as mg YTX eq./kg shellfish flesh.

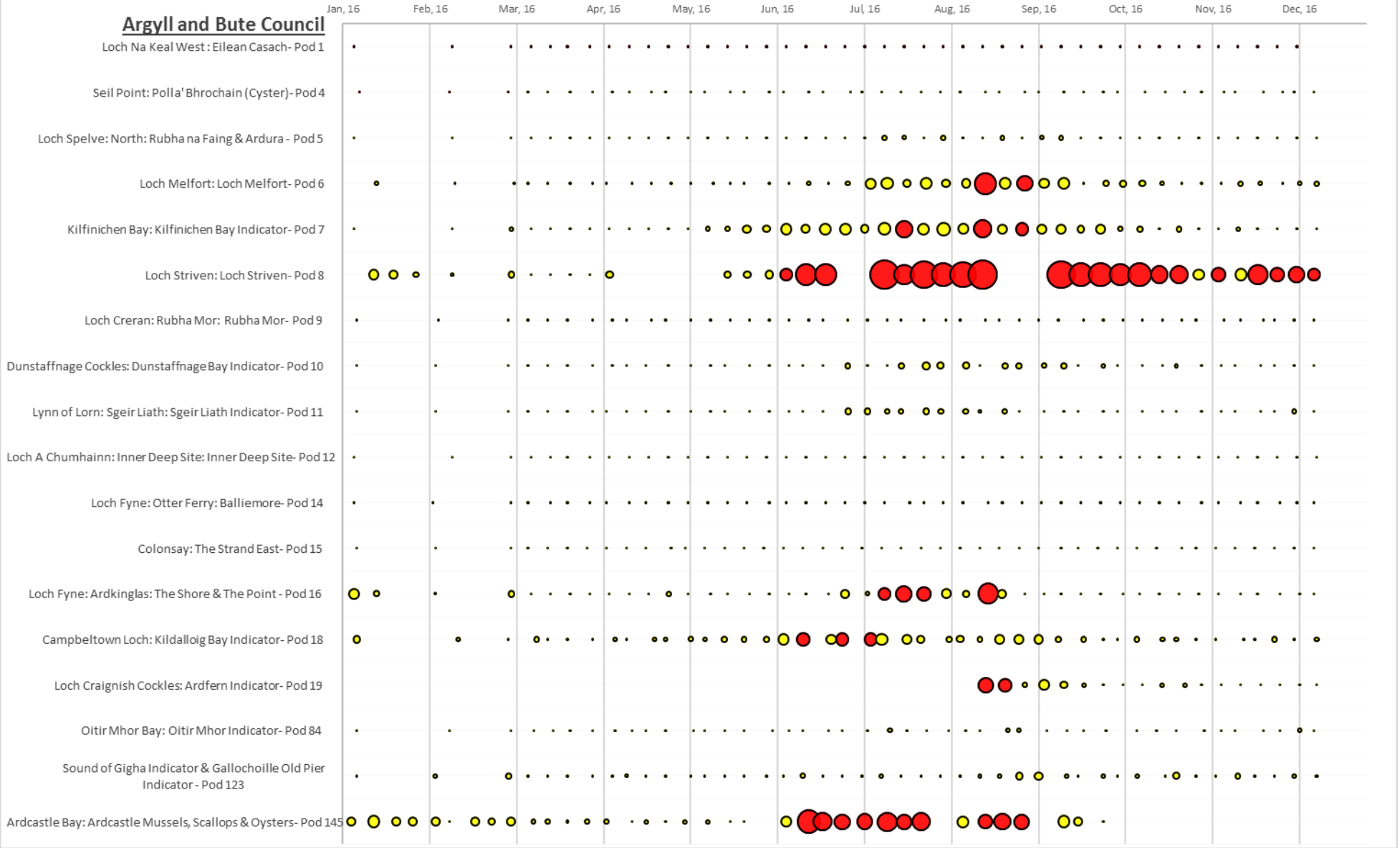
During this reporting period, 191 inshore samples breached maximum permitted levels (MPL) for lipophilic toxins. As observed previously, where monitoring for lipophilic toxins had occurred in the previous two weeks, the LC-MS method provided an early warning, detecting low toxin levels either one or two weeks prior to closure in all bar one instance, indicating the methods performance and advantage as an early warning mechanism, when applied to risk management practices such as the FSS "traffic light" guidance.

In total, lipophilic toxins analyses were performed on 2,841 samples from inshore locations and 28 verification samples collected from commercial establishments. Results are summarised below.

OA/DTX/PTX group

- OA/DTX/PTX group toxins were detected in 801 inshore samples, comprising of mussels (735 samples), Pacific oysters (14), common cockles (7), surf clams (17), razors (15) and Queen scallops (13).
- OA/DTX/PTX group toxins were detected in all months throughout the reporting period (Figure 3), with the majority of recorded results occurring between June and November 2016 (700 samples).
- The distribution of OA/DTX/PTX toxins was widespread, affecting sites within all monitored local authority regions.
- One hundred and ninety-one samples comprising of mussels (174 samples), Pacific oysters (4), Queen scallops (10) and Surf clams (3) from 34 sites recorded results above the MPL. All above MPL results were recorded between May and December 2016 (Figure 4).
- The highest level recorded during 2016 was 2,660µg OA eq./kg, more than sixteen times the regulatory limit, in a sample from Loch Laxford (Highland: Sutherland) in late July 2016. Levels of OA/DTX/PTX group toxins at this site rose from 51µg OA eq./kg to 501µg OA eq./kg within a two-week period.
- Elsewhere, OA/DTX/PTX group toxins were detected below the MPL in a further 610 samples from 71 sites (Figure 5), between January and December 2016.
- YTX group toxins were detected in 16 samples which contained OA/DTX/PTX group above the MPL between June, July and August 2016. None of these samples exceeded the YTX group MPL. A further 13 samples were found to contain YTX and OA/DTX/PTX group toxins below the relative MPLs between April and September 2016.
- OA/DTX/PTX group toxins below the MPL were detected in one whole king scallop verification samples from the Clyde 02 scallop grounds received in February 2016.

Figure 3: Concentrations of OA/DTX/PTX group toxins from January to December 2016



Concentration of OA/DTX/PTX group toxins:

2,000 µg OA eq/kg

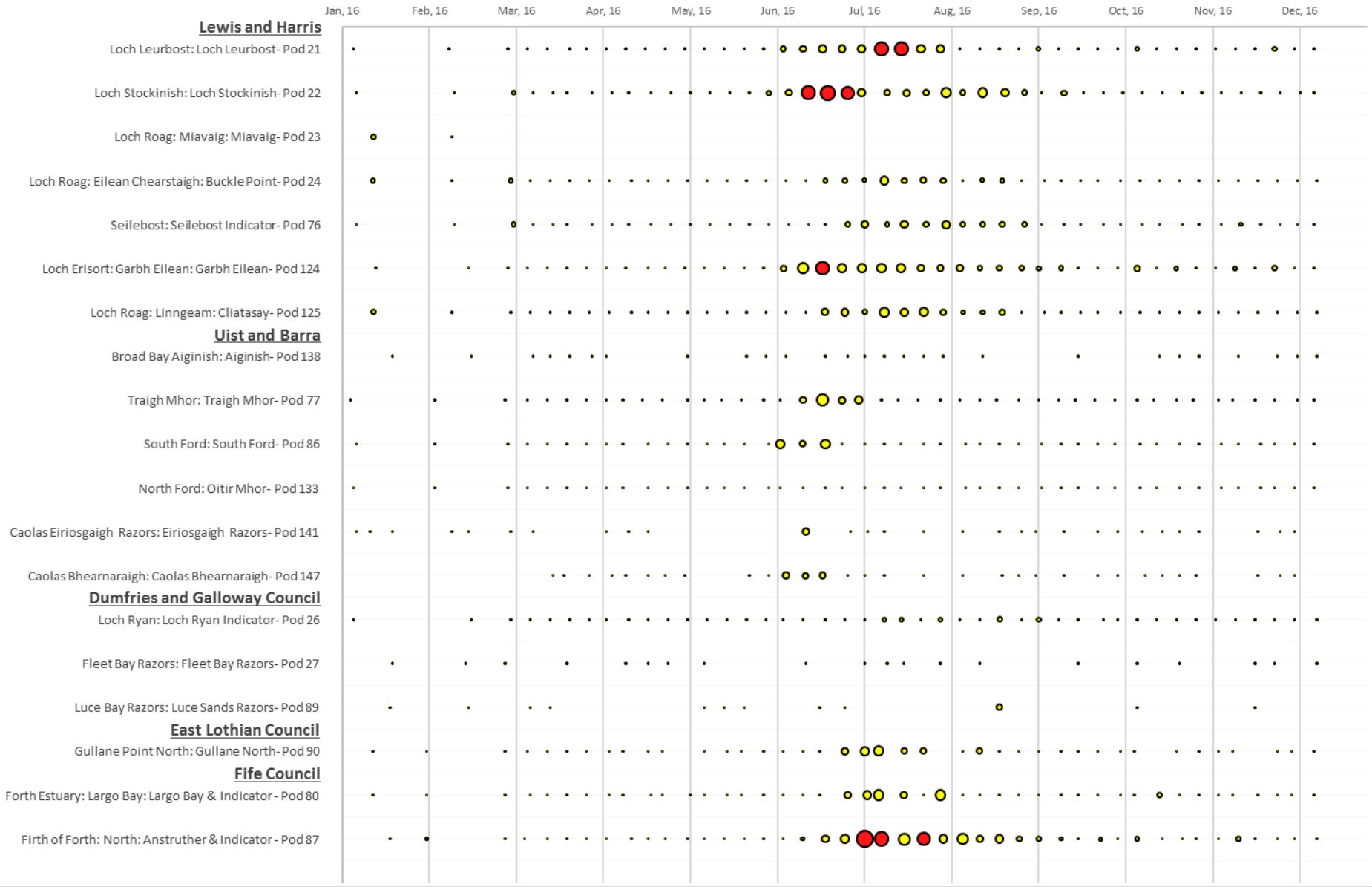
1,000 µg OA eq/kg

>160 µg OA eq/kg (MPL)

• Not Detected

Red = Toxins present above MPL
Yellow = Toxins present below MPL

Figure 3: Concentrations of OA/DTX/PTX group toxins from January to December 2016 (continued)



Concentration of OA/DTX/PTX group toxins:

2,000 µg OA eq/kg

1,000 µg OA eq/kg

>160 µg OA eq/kg (MPL)

• Not Detected

Red = Toxins present above MPL
Yellow = Toxins present below MPL

Figure 3: Concentrations of OA/DTX/PTX group toxins from January to December 2016 (continued)

Concentration of OA/DTX/PTX group toxins:

2,000 µg OA eq/kg

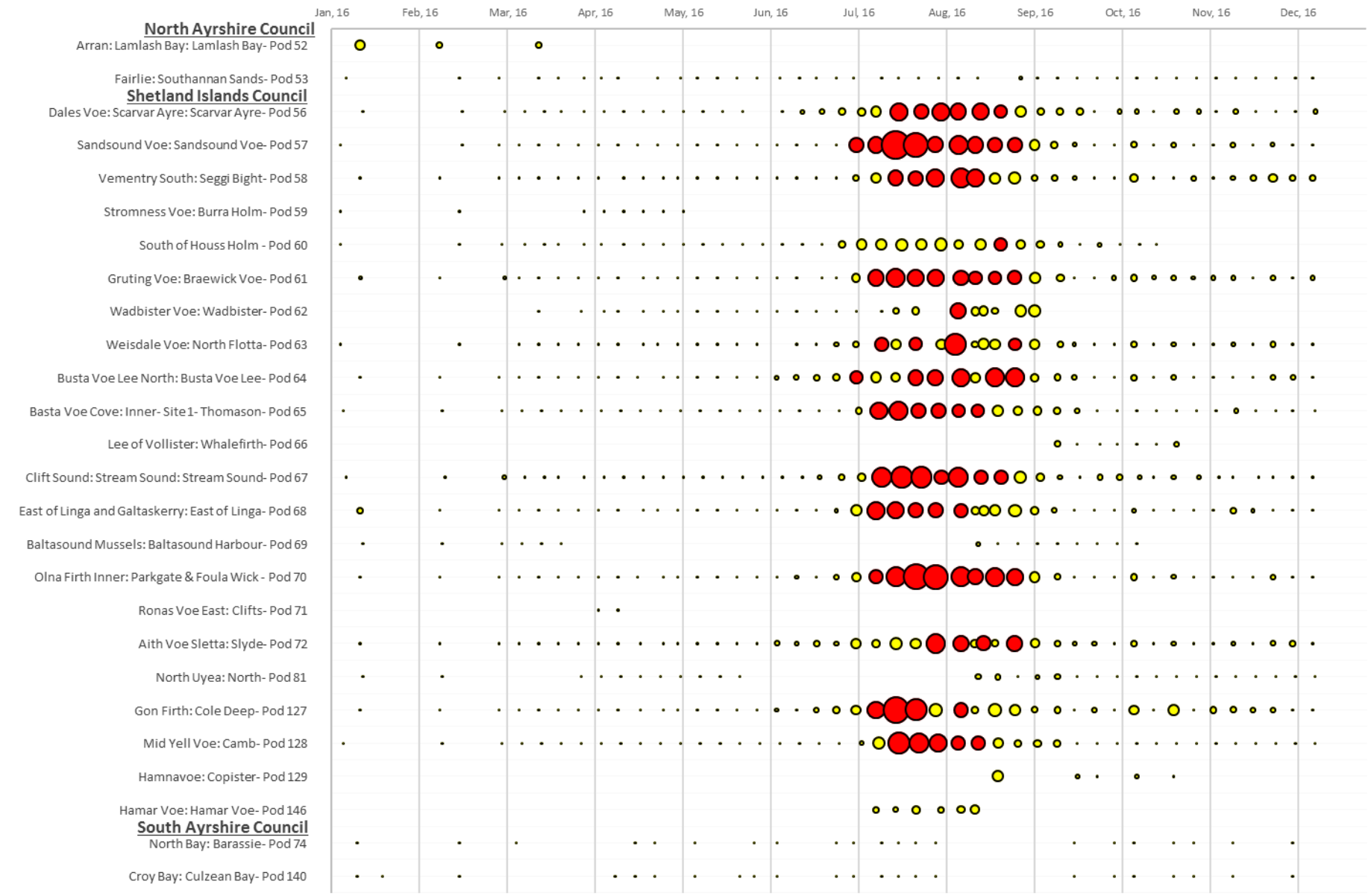
1,000 µg OA eq/kg

>160 µg OA eq/kg (MPL)

Not Detected

Red = Toxins present above MPL
Yellow = Toxins present below MPL

Figure 3: Concentrations of OA/DTX/PTX group toxins from January to December 2016 (continued)



Concentration of OA/DTX/PTX group toxins:

2,000 µg OA eq/kg

1,000 µg OA eq/kg

>160 µg OA eq/kg (MPL)

• Not Detected

Red = Toxins present above MPL
Yellow = Toxins present below MPL

Figure 4: Inshore locations recording OA/DTX/PTX group results above the maximum permitted limit (>160µg OA eq./kg) in 2016

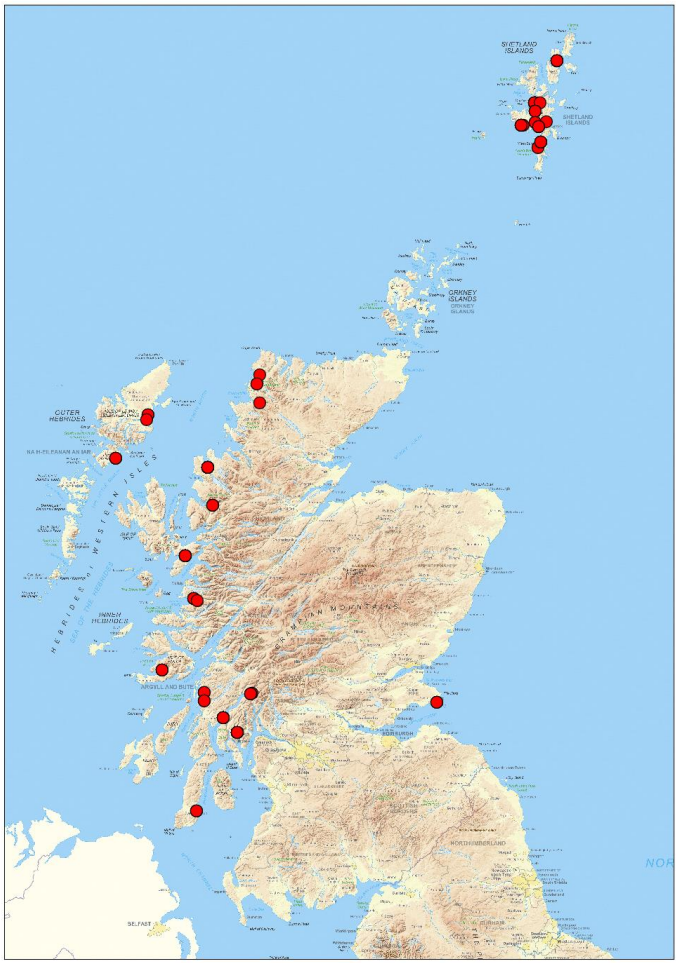
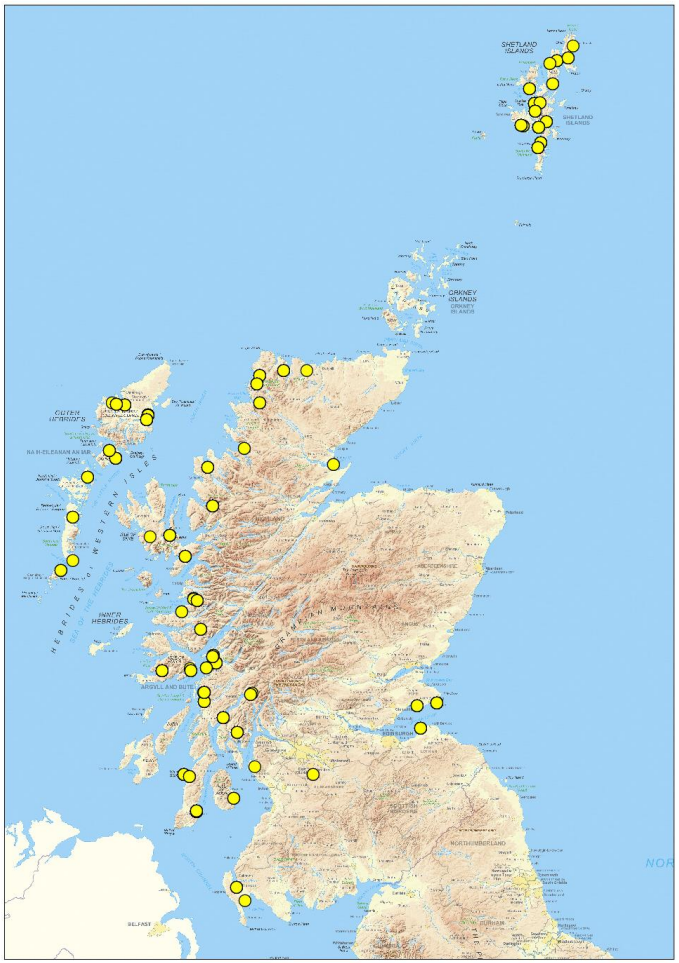


Figure 5: Inshore locations where toxins of OA/DTX/PTX group were detected below the maximum permitted limit (≤160µg OA eq./kg) in 2016



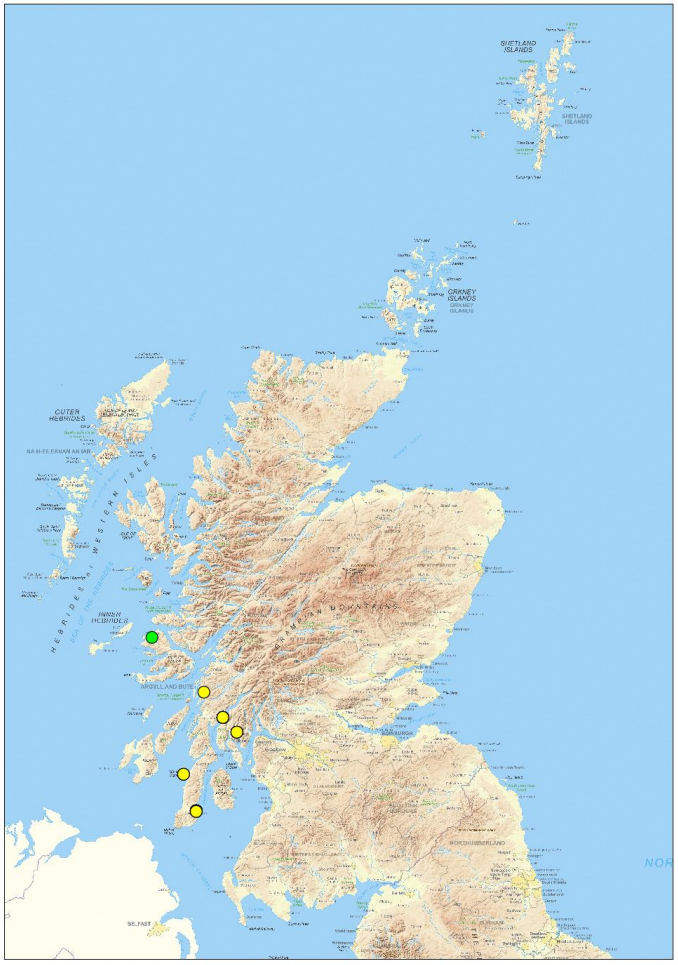
AZA group

- AZAs were detected in one sample in 2016, from Loch A Chumhainn at 22µg AZA eq./kg, in early December (Figure 6).

YTX group

- YTXs were detected in 29 mussel samples from 6 sites during the reporting period.
- The occurrence of YTX toxins in 2016 was limited to sites within the Argyll & Bute region.
- Results from 2011, 2012, 2015 & 2016 indicated the distribution of the YTX group was fairly localised, predominantly within the Loch Fyne, Firth of Clyde and Firth of Lorn regions. Results from 2013 & 2014 indicated a geographic shift in the distribution of YTXs, with the above regions and Shetland Isles also being affected.
- YTXs were detected throughout the year, and were largely prevalent between June and August 2016, during which time they were detected in 25 of the 29 samples containing YTX group toxins.
- No samples exceeded the MPL (3.75mg YTX eq./kg) in 2016, which is consistent with previous years. **The highest level recorded was 1.9mg/kg in one sample of Queen scallops** from Ardcastle Bay Scallops: Ardcastle Scallops (Argyll & Bute) in mid-June. This RMP was monitored briefly from mid-June to mid-September 2016, of the 14 samples submitted 13 contained YTX group toxins (one perished prior to arrival at the lab and was not tested). The remaining samples recorded results between 0.2 and 0.5mg/kg (Figure 6).
- OA/DTX/PTX group toxins above the MPL were detected in 16 samples (6 mussel & 10 Queen scallop samples), which also contained YTXs below the MPL (see above). OA/DTX/PTX group toxins below the MPL were also detected in 13 samples where YTX group toxins were present below the MPL.
- YTX toxins were not detected in any of the King scallop verification samples

Figure 6: Inshore locations where toxins of the AZA group ( ) YTX group ( )
were detected below the maximum permitted level (≤160µg AZA eq./kg & ≤3.75mg YTX eq./kg) in 2016



Phytoplankton associated with the production of lipophilic toxins

- *Dinophysis* spp.* were present in 576 samples (44.27%) analysed during 2016 and were detected from March to October (Figure 7).
- They were observed at or above trigger level (set at 100 cells/L) in 257 samples (19.75%) between April and September.
- The earliest bloom** exceeding trigger level was recorded in Loch Striven (Argyll & Bute) in early April.
- Two exceptionally dense blooms were observed during 2016. An abundance of 24,340 cells/L was recorded in Loch Ewe (Highland: Ross & Cromarty) on 6th June and 90,274 cells/L in Loch Fyne: Ardkinglas (Argyll & Bute) on 22nd June.
- The majority of *Dinophysis* spp. blooms occurred around the Scottish coast in June and July, with 55.63% of the samples exceeding threshold counts in July.
- *Dinophysis* spp. blooms were widespread around Argyll & Bute, and the Highland region from late May to early September, with associated DSP toxicity reported in shellfish. Toxic blooms also occurred in Loch Stockinish and Loch Erisort (Lewis & Harris) in June. Blooms of *Dinophysis* spp. were recorded around the Shetland Islands from June to mid-August with widespread toxicity throughout July and August.
- Overall, the percentage of *Dinophysis* spp. blooms at or exceeding trigger level during the current reporting period (19.75%) was very similar to that in both 2014 (19.29%) and 2015 (19.30%).

*references to *Dinophysis* spp. in this report also include *Phalacroma rotundatum* (synonym *Dinophysis rotundata*)

** blooms are denoted as cell counts at or exceeding trigger level, where appropriate for individual species/genera.

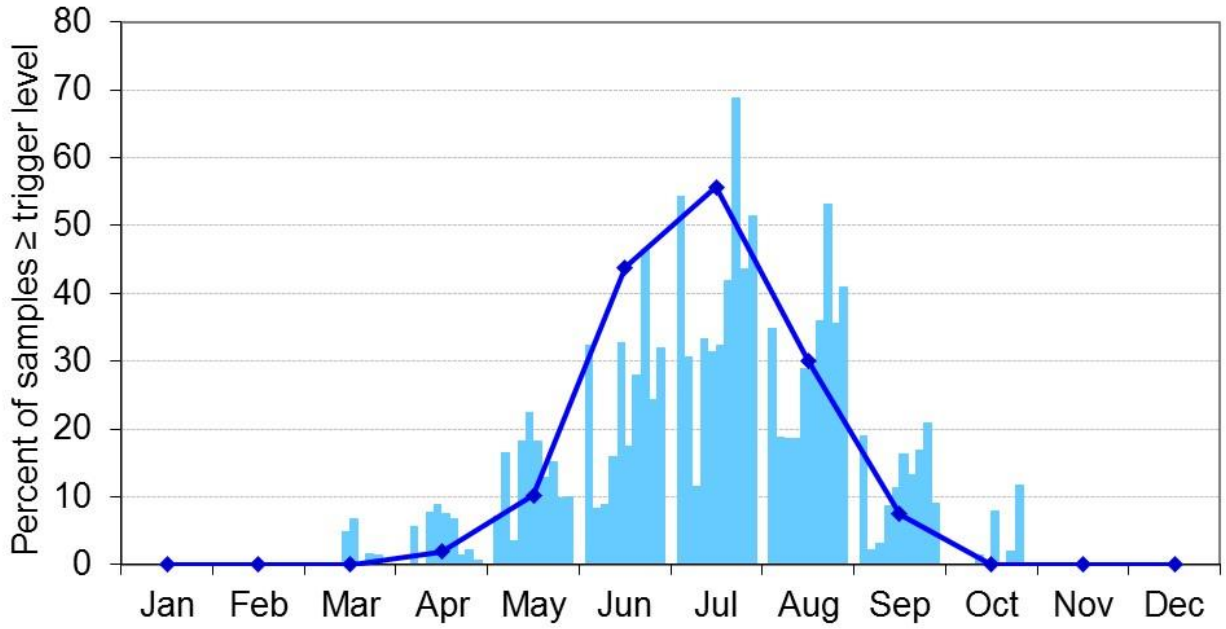


Figure 7: The percentage of samples in which *Dinophysis* spp. equalled or exceeded the trigger level of 100 cells/L in 2016 is indicated by the line. (For comparison, the bars show the percentage of samples in which *Dinophysis* cells equalled or exceeded the trigger level between 2006 and 2015**).**

Figure 8: *Dinophysis* species observed on 18th July in Loch Inchard (Highland: Sutherland).

- *Prorocentrum lima* was present in 228 samples (17.52%) analysed during 2016. It was recorded from March to December, and was generally most abundant in July. It was detected at or above the trigger level (set at 100 cells/L) in 30 samples (2.31%) between May and September. *Prorocentrum lima* was observed in samples from all regions and the densest blooms recorded in 2016 were 900 cells/L at Kyle of Tongue (Highland: Sutherland) and 780 cells/L at Loch Fyne: Otter Ferry (Argyll & Bute), both on 1st June.

- *Protoceratium reticulatum* was detected in 37 samples (2.84%) between March and September, and was most frequently observed in May and June. The densest bloom occurred in Argyll & Bute, with 1,360 cells/L recorded at Loch Fyne: Ardkinglas on 22nd June, although no YTX toxicity was detected in Pacific oysters from this site. However, low levels of YTX toxins were detected in common mussels from Campbeltown Loch, Loch Striven and Loch Melfort (Argyll & Bute) when *Protoceratium reticulatum* was present.

- *Lingulodinium polyedrum* is rarely abundant in Scottish coastal waters and was detected on only three occasions (0.23 % of samples), all in Argyll & Bute during 2016. One observation was recorded in Loch Creran in September, where it appears to bloom annually. It was also reported in Kilfinichen Bay in May. The maximum bloom density of 200 cells/L was observed in Loch Na Keal on 7th June.

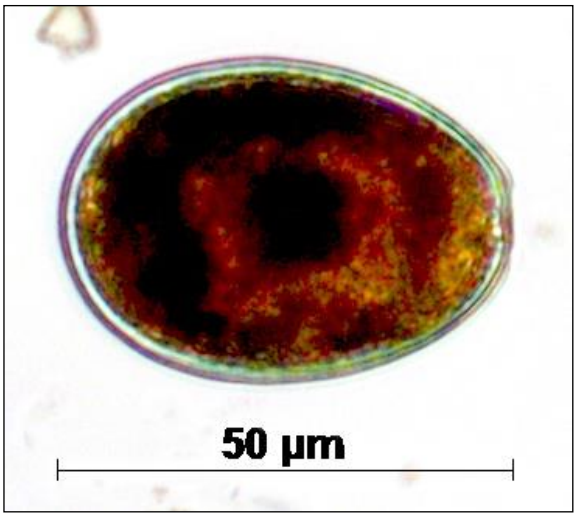


Figure 9: *Prorocentrum lima* observed at Colonsay: The Strand East (Argyll & Bute) on 15th August.

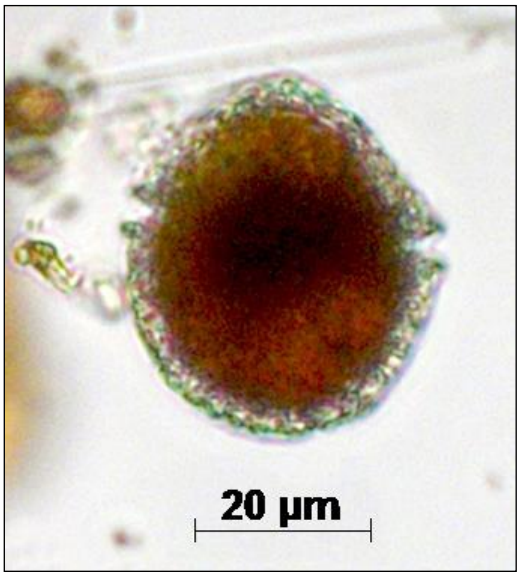
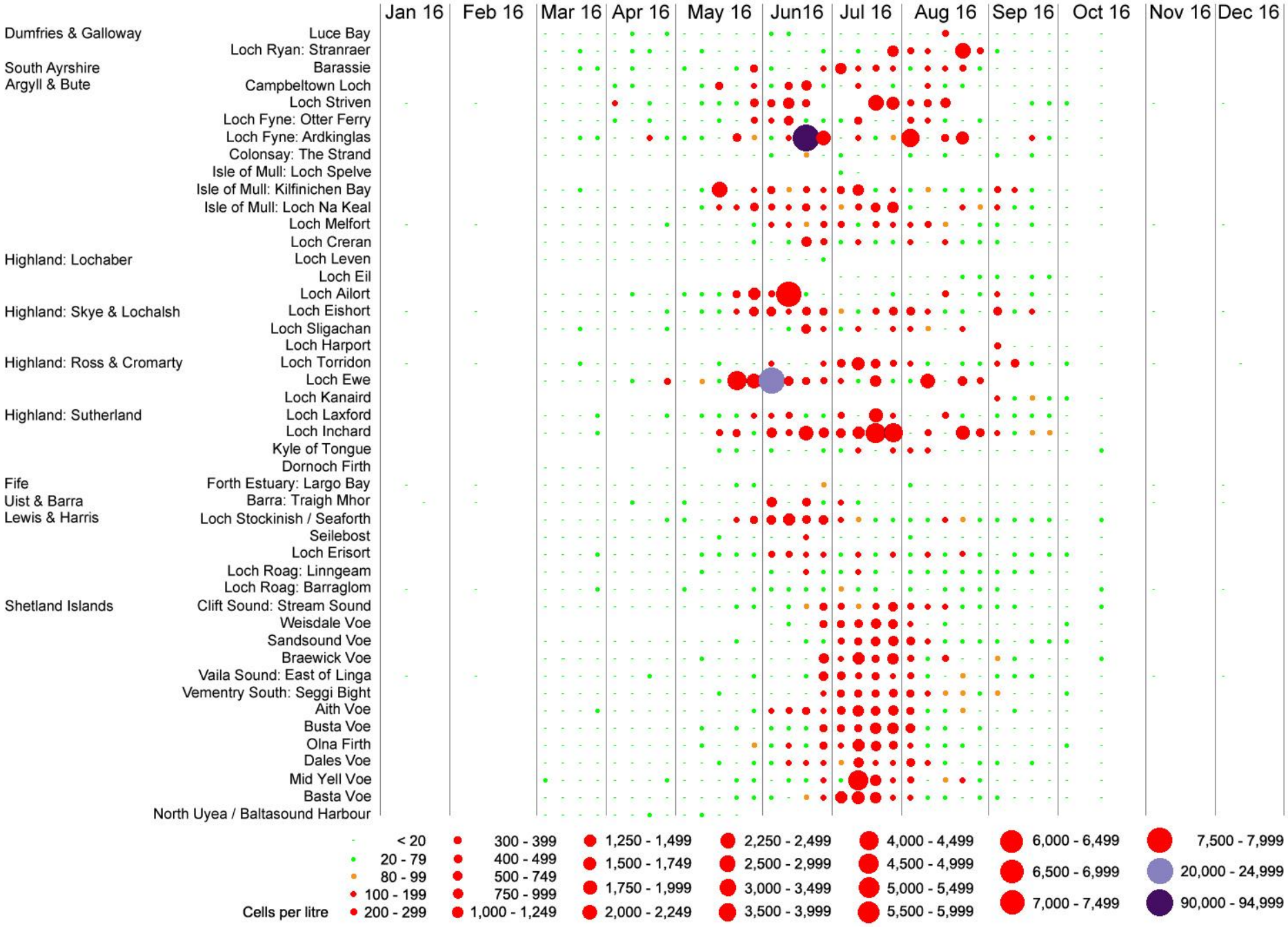


Figure 10: *Protoceratium reticulatum* was observed in Loch Melfort (Argyll & Bute) on 17th of August).

Figure 11: *Lingulodinium polyedrum* was observed in Loch Creran (Argyll & Bute) on 5th September.

Figure 12. Phytoplankton concentrations of *Dinophysis* spp. observed between January and December 2016

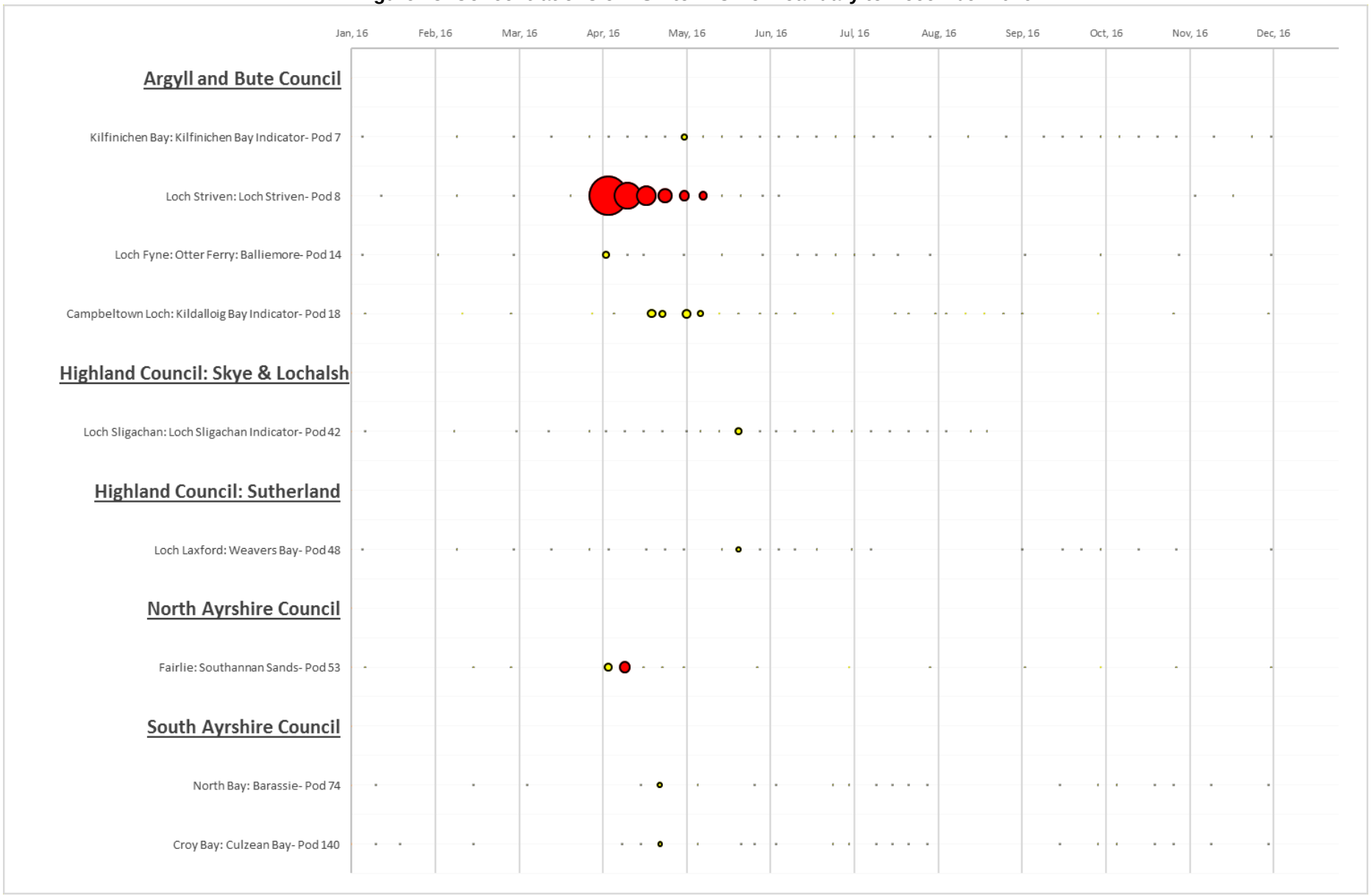


Monitoring for PSP toxins

A total of 1,717 samples from inshore locations and 28 king scallop verification samples collected from commercial establishments were tested for paralytic shellfish poisoning (PSP) toxins. All samples were tested by a high performance liquid chromatography (HPLC) method, and are summarised below.

- Five mussel samples from Loch Striven and one Pacific oyster sample from Fairlie: Southannan Sands (both in Argyll & Bute, Figure 14) were found to contain PSP toxins above the MPL of 800µg STX eq./kg shellfish flesh between April and early May 2016. The highest level recorded was 11,043µg/kg, over thirteen times the regulatory limit in a mussel sample from Loch Striven (Argyll & Bute) collected in early April 2016.
- PSP toxins above reporting levels, but below the MPL were detected in a further 12 samples comprising of mussels (8) and Pacific oysters (2) from 9 sites (Figure 15). All occurrences were recorded between April and May 2016.
- Results from 2008 to 2015 indicated that PSP toxicity episodes began typically in March/April and predominantly conclude by July or August. The 2016 PSP season (Figure 13), concluding by mid to late May represents one of the shortest seasons since the introduction of the HPLC testing method. This is in stark contrast to the 2015 PSP season which continued until mid-September.
- A range of PSP toxins were quantified during 2016, with profiles predominantly consisting of the toxins STX, GTX2&3, GTX1&4, NEO and C1&2 (data not shown). Lower concentrations of GTX5 and dcSTX were also detected in shellfish samples. Proportions of each toxin varied considerably, but the profiles were consistent with previous years, and similar to those expected from shellfish contaminated with *Alexandrium* as documented in Turner *et al*, 2014. Interestingly, the two PSP positive razor clam samples exhibited a slightly different toxin profile to other samples, being predominantly composed of STX and GTX2&3 with only low concentrations of NEO and GTX1&4.
- One king scallop verification sample (shucked product sample), originating from the East 3 scallop ground in May 2016, contained PSP toxins below the regulatory limit (395µg STX eq./kg).

Figure 13. Concentrations of PSP toxins from January to December 2016



Concentration of PSP toxins:

10,000µg STX eq/kg

5,000µg STX eq/kg

800µg STX eq/kg (MPL)

Not Detected

Red = Toxins above MPL
Yellow = Toxins below MPL

Figure 14: Inshore locations recording PSP toxin results above the maximum permitted limit (>800µg STX eq./kg) in 2016

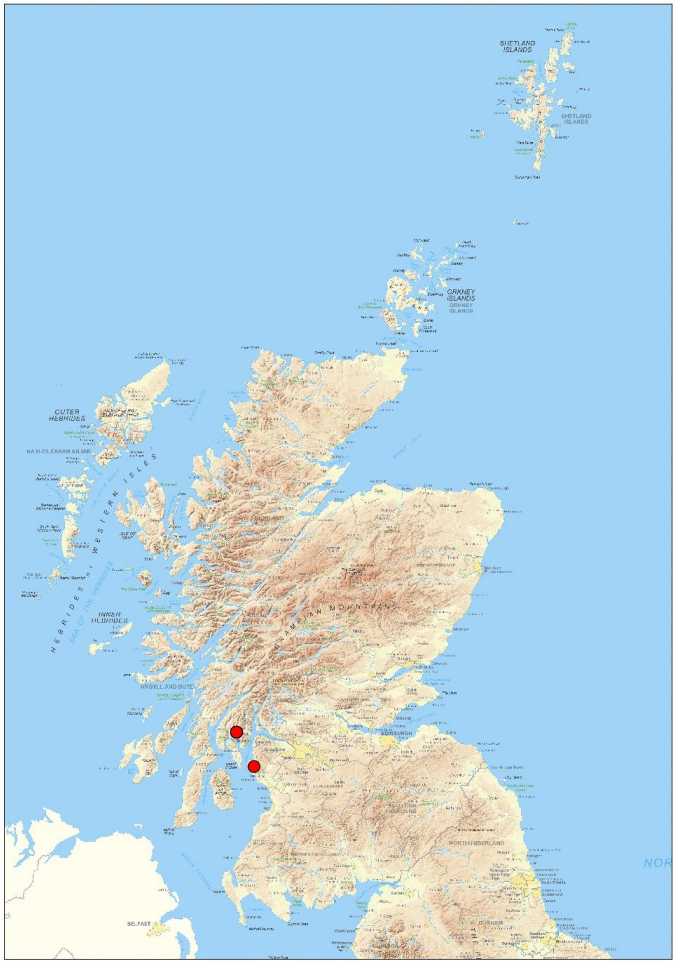
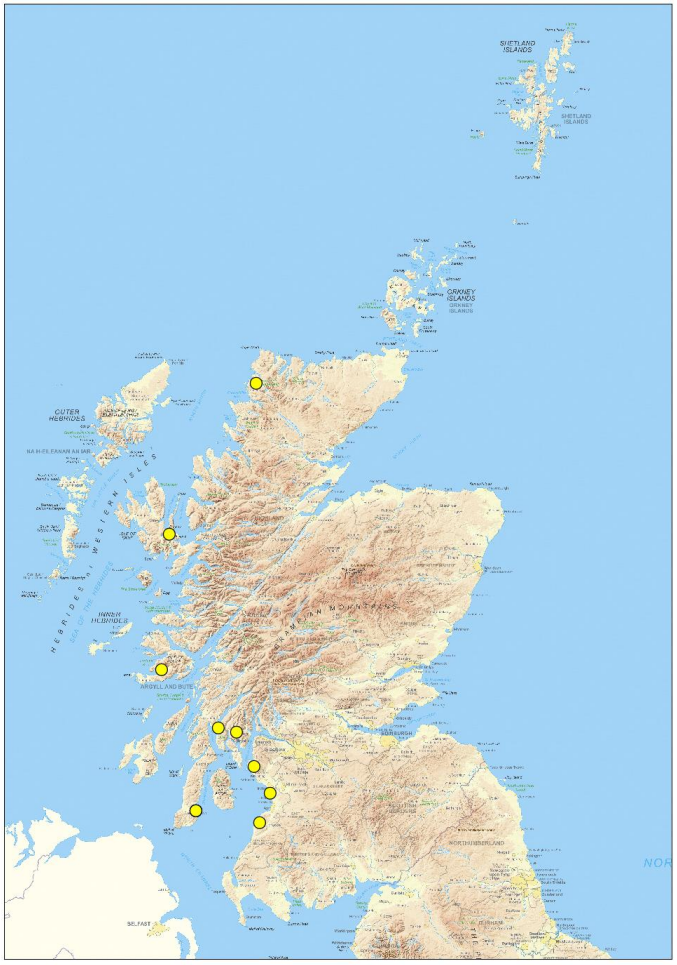


Figure 15: Inshore locations recording PSP toxin results below the maximum permitted limit (≤800µg STX eq./kg) in 2016



Phytoplankton associated with the production of PSP toxins

- *Alexandrium* spp. were observed between March and October (Figure 16) and were detected in 421 samples (32.36%) analysed during 2016. They were reported at or above the trigger level (set at 40 cells/L) in 280 samples (21.52%) mostly between May and July, and were recorded at or exceeding trigger level in 44.38% of the samples analysed during July.
- The densest recorded *Alexandrium* spp. bloom was observed in Loch Creran (Argyll & Bute) on 18th July with an abundance of 5,860 cells/L, although no PSP toxicity was detected in Pacific oysters from this site.
- Toxic *Alexandrium* spp. blooms were detected in other areas around Argyll & Bute during spring, most notably Campbeltown Loch (440 cells/L on 11th April), Loch Striven (840 cells/L on 5th April), Loch Fyne: Otter Ferry (240 cells/L on 4th April), and Kilfinichen Bay (320 cells/L on 26th April). Some PSP toxicity was also associated with *Alexandrium* recorded in the Highland region in May, around Loch Sligachan (Skye & Lochalsh) and Loch Laxford (Sutherland).
- Overall, the percentage of *Alexandrium* spp. blooms at or exceeding trigger level during 2016 (21.52%) was very similar to that in 2015 (21.67%).

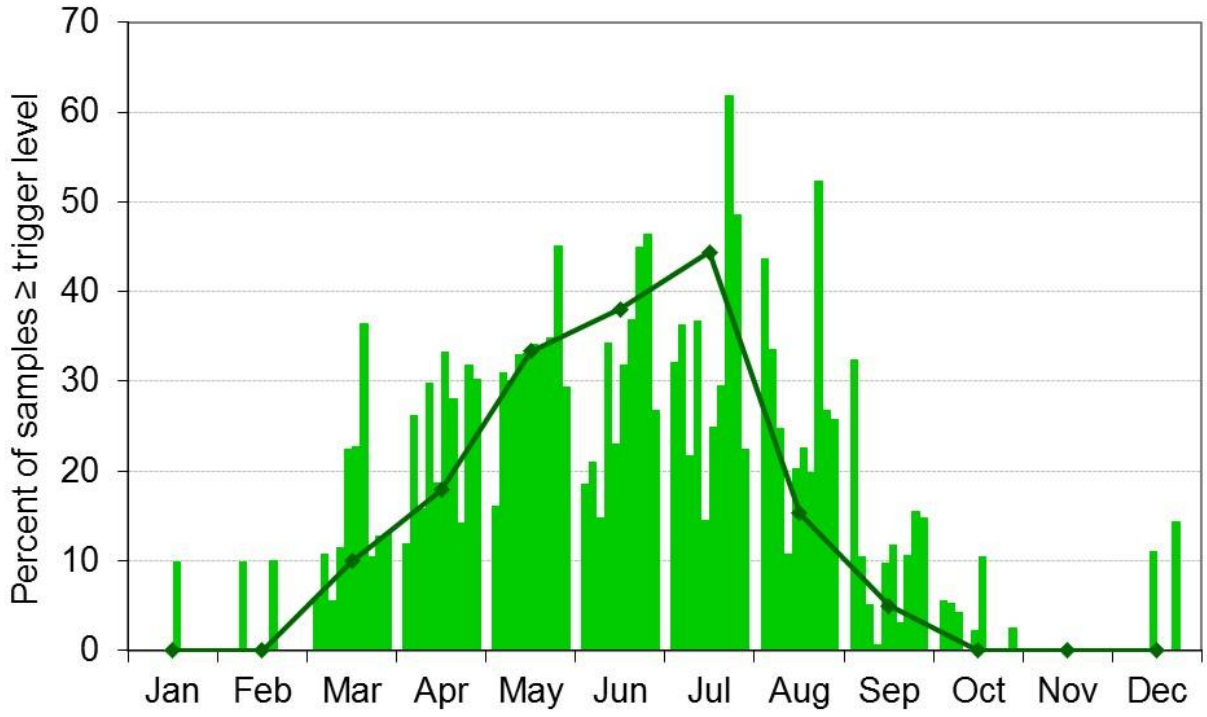


Figure 16: The percentage of samples in which *Alexandrium* spp. equalled or exceeded the trigger level of 40 cells/L in 2016 is indicated by the line.
(For comparison, the bars show the percentage of samples in which *Alexandrium* spp. equalled or exceeded the trigger level between 2006 and 2016. NOTE: Data collected prior to July 2014 have been adjusted to the revised trigger level of 40 cells/L for comparative purposes).

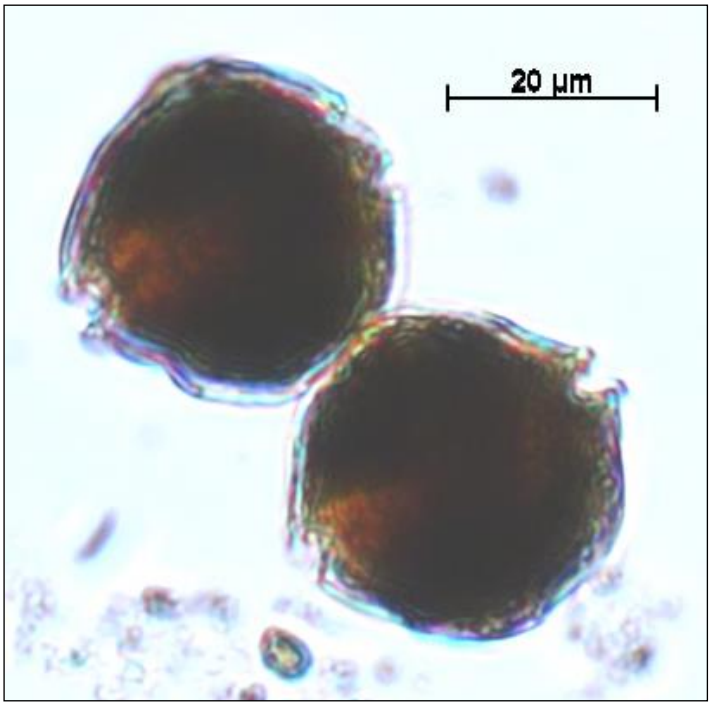
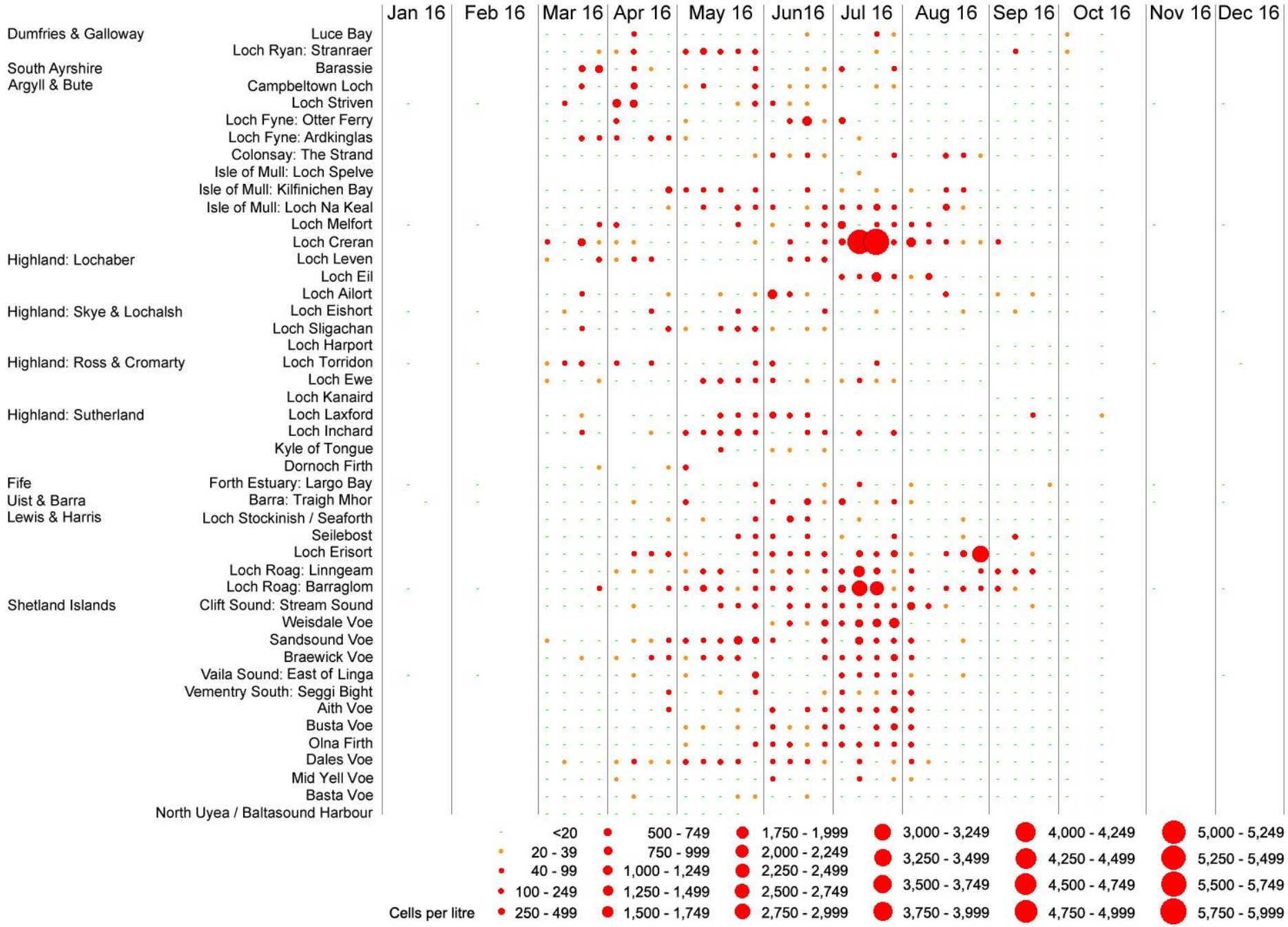


Figure 17: *Alexandrium* spp. observed at Barassie (South Ayrshire) on 30th March.

Figure 18. Phytoplankton concentrations of *Alexandrium* spp. observed between January and December 2016

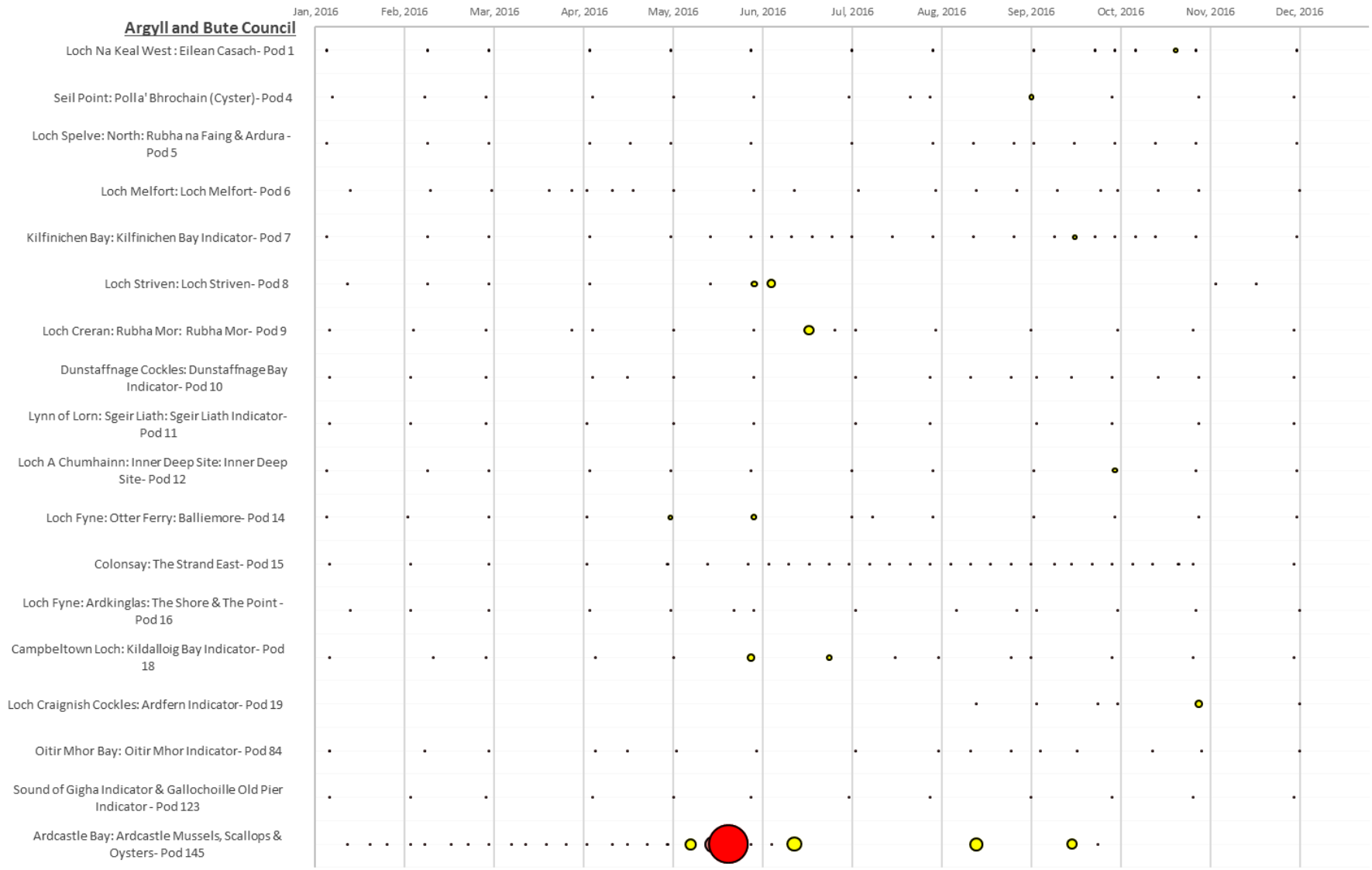


Monitoring for ASP toxins

Analyses for amnesic shellfish poisoning (ASP) toxin were conducted on 1,277 samples from inshore locations and 28 king scallop verification samples collected from commercial establishments. All samples were analysed by an HPLC method. Results are summarised below.

- ASP was detected in 89 inshore samples comprising of: common mussels (28 samples), razors (14), Pacific oysters (17), common cockles (9), surf clams (15) and Queen scallops (3).
- These samples originated from 41 sites. Low concentrations were recorded from March through to December 2016, with the peak period occurring between May & September, during which time, ASP was detected in 76 samples (Figure 19).
- Four inshore samples exceeded the MPL of 20mg [domoic/epi domoic acid] (DA)/kg shellfish flesh (Figure 20). **The highest level recorded was 49mg/kg in a mussel sample collected in mid May 2016,** originating from Ardcastle Bay Mussels: Ardcastle Mussels (Argyll & Bute). **This is the highest result recorded from an inshore location since 2008.** The remaining 3 results, which exceeded the MPL, were all detected in razor samples in early to mid-June; 2 samples from South Ayrshire (North Bay: Barassie – 24mg/kg and Croy Bay: Culzean Bay – 29mg/kg) and 1 from Uist and Barra (Caolas Eiriosgaigh – 34mg/kg).
- ASP below the action limit was detected in a further 85 samples (Figure 21). Results ranged from 1mg/kg up to 15mg/kg, with 69 (81.2%) of these samples below 5mg/kg. The period where ASP was detected in 2016 marginally longer than previous years, concluding in December. In previous years ASP has not been detected beyond October or November. The number of recorded occurrences is consistent with previous years.
- ASP was detected in six king scallop verification samples from 5 establishments. Four of these samples comprised of whole king scallop material, the remaining two of shucked product. These shellfish samples were originally harvested in the following offshore scallop grounds; Jura (4 samples), Clyde (1 samples), South Minch (1 sample). Toxin levels ranged between 1.7 and 39mg/kg DA/shellfish flesh, with one whole scallop sample from the Clyde exceeding the MPL.

Figure 19: Concentrations of ASP toxins from January to December in 2016

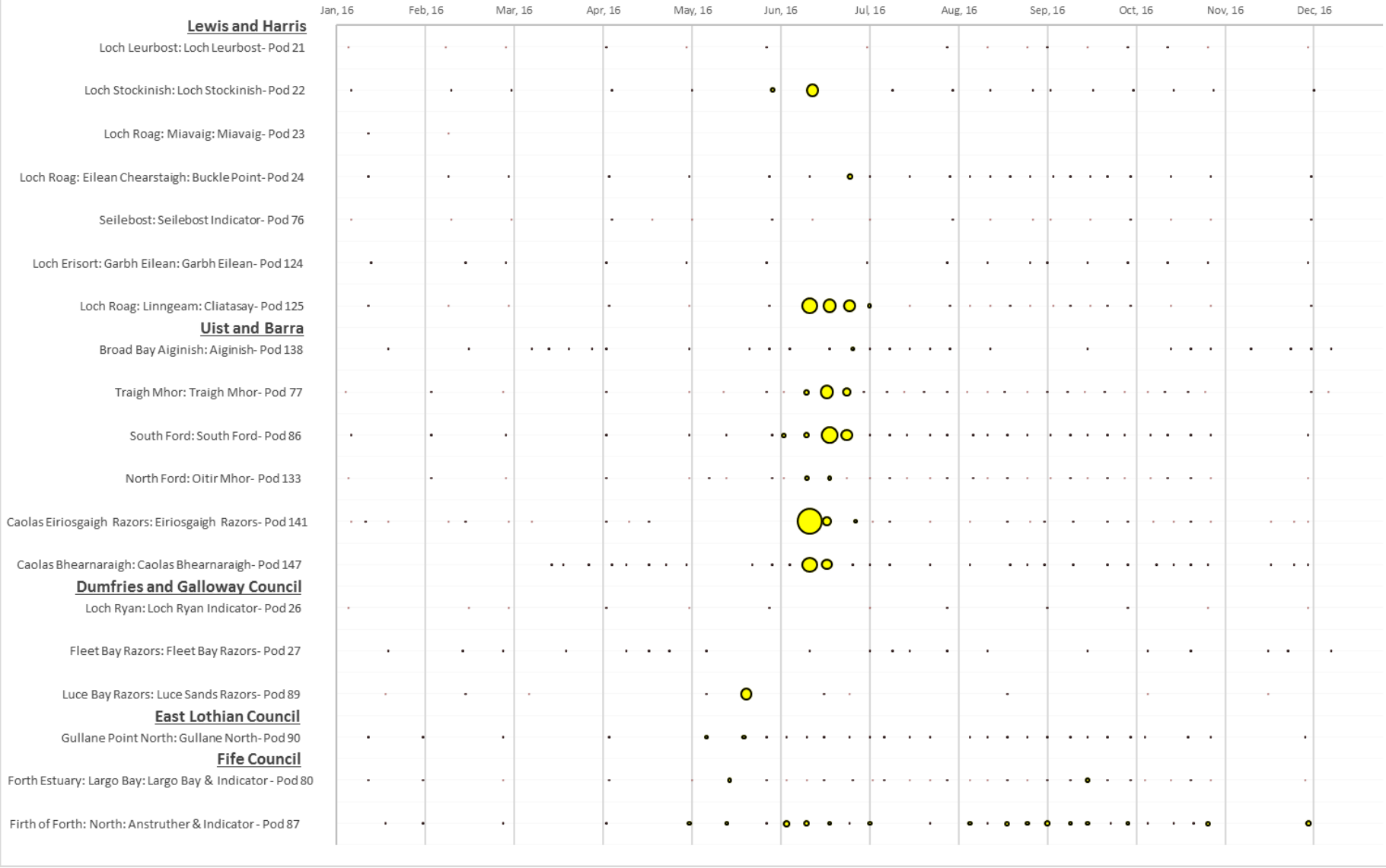


Concentration of ASP toxins:

20mg/kg ASP (MPL) | 10mg/kg ASP | 5mg/kg ASP | • Not Detected

Red = Toxins above MPL
Yellow = Toxins below MPL

Figure 19: Concentrations of ASP toxins from January to December in 2016

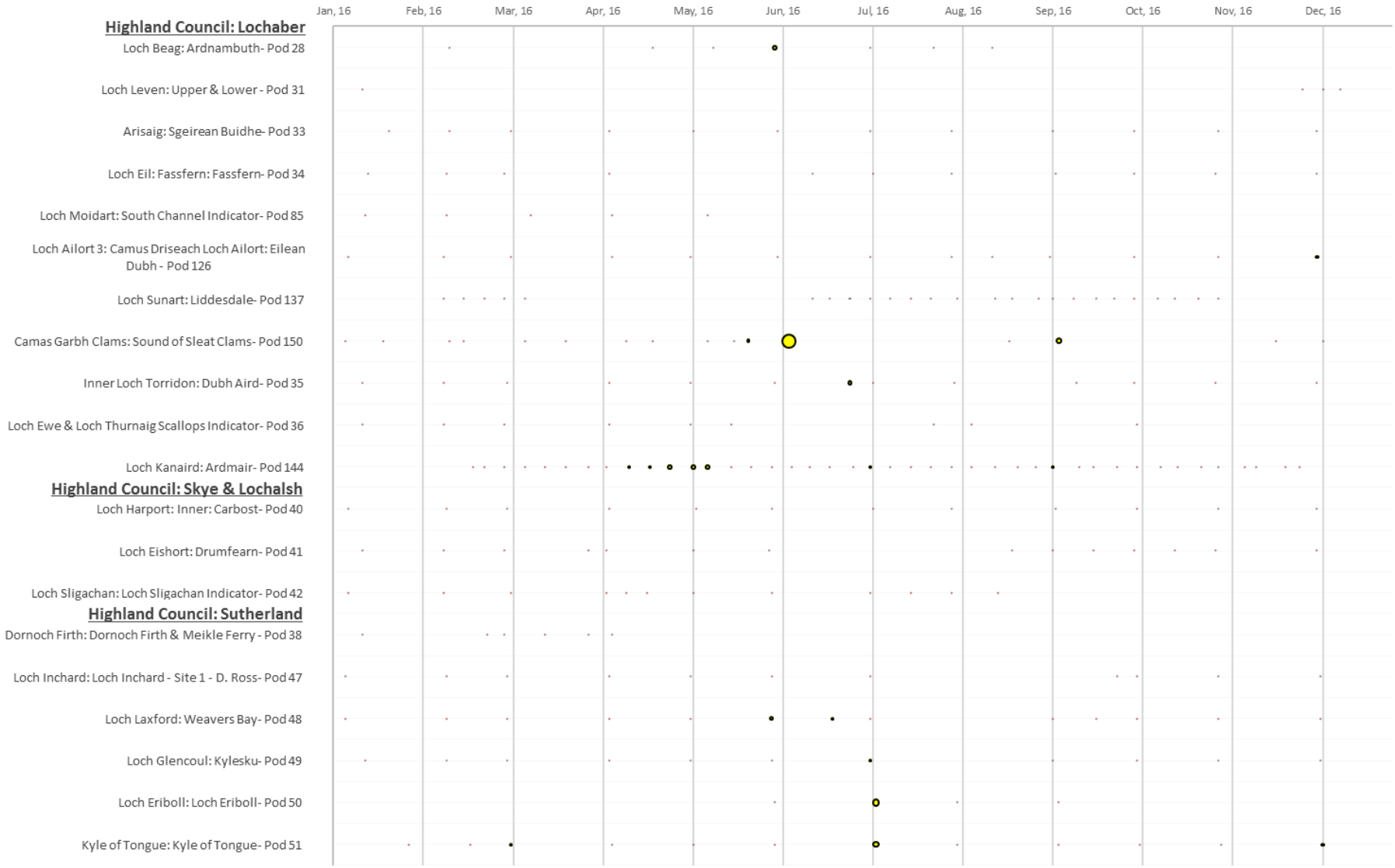


Concentration of ASP toxins:

20mg/kg ASP (MPL) | 10mg/kg ASP | 5mg/kg ASP | • Not Detected

Red = Toxins above MPL
Yellow = Toxins below MPL

Figure 19: Concentrations of ASP toxins from January to December in 2016

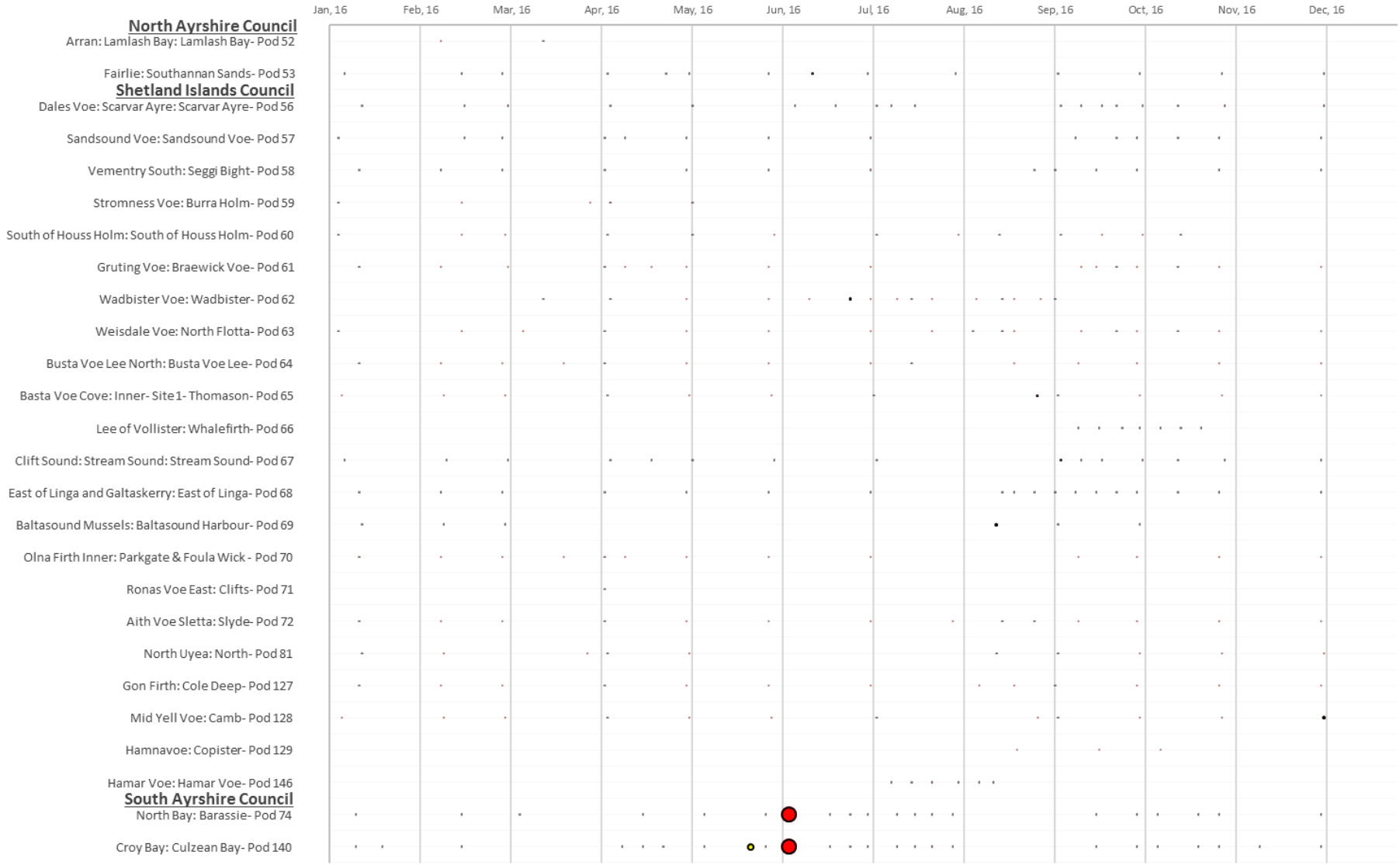


Concentration of ASP toxins:

20mg/kg ASP (MPL) | 10mg/kg ASP | 5mg/kg ASP | Not Detected

Red = Toxins above MPL
Yellow = Toxins below MPL

Figure 19: Concentrations of ASP toxins from January to December in 2016

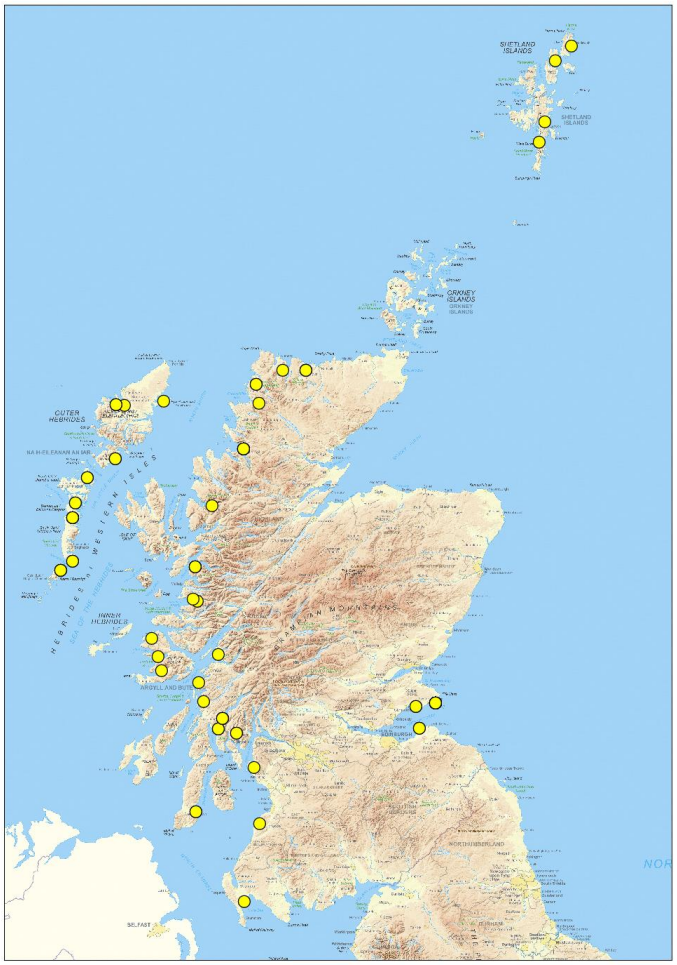


Concentration of ASP toxins:

20mg/kg ASP (MPL) | 10mg/kg ASP | 5mg/kg ASP | Not Detected

Red = Toxins above MPL
Yellow = Toxins below MPL

Figure 21: Inshore locations where ASP toxins were detected below the maximum permitted limit (≤20mg/kg) in 2016



Phytoplankton associated with the production of ASP toxins

- *Pseudo-nitzschia* spp. were detected every month in 2016 (Figure 22) and at all sites, and were present in 1,193 (91.70%) of the samples analysed. Blooms (here referred to as cell densities exceeding 50,000 cells/L) were detected between March and October, and were most frequently observed in March, with relatively few being detected in May.
- *Pseudo-nitzschia* spp. counts at or above the trigger level (set at 50,000 cells/L) were recorded in 80 samples (6.15%), with 10.00% of the samples analysed in March exceeding this level. The earliest bloom was recorded in Dornoch Firth (Highland: Sutherland) on 7th March, with an abundance of 245,770 cells/L. A late bloom occurred around the Isle of Mull (Argyll & Bute) in October and was detected in both Kilfinichan Bay and Loch Na Keal.
- The densest *Pseudo-nitzschia* spp. bloom was observed in Loch Roag: Linngeam (Lewis & Harris) on 21st June, where a maximum abundance of 1,127,883 cells/L was recorded. Some associated ASP toxicity was reported in common mussels from this site during the bloom period. Most of the blooms associated with ASP toxicity in 2016 occurred in May and June.
- Overall, the percentage of *Pseudo-nitzschia* spp. blooms at or exceeding trigger level during 2016 (6.15%) was lower than that in 2015 (8.88%) or 2014 (9.13%), but was more similar to 2013 (6.54%).

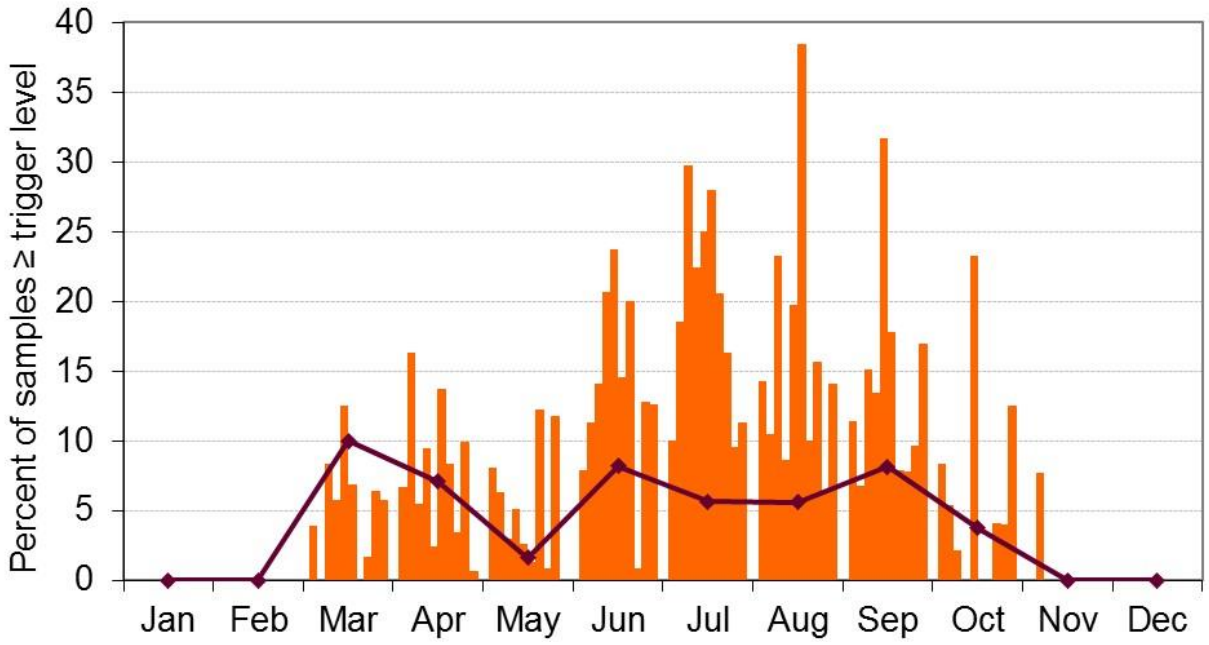


Figure 22: The percentage of samples in which *Pseudo-nitzschia* spp. equalled or exceeded the trigger level of 50,000 cells/L in 2016 is indicated by the line. For comparison, the bars show the percentage of samples in which *Pseudo-nitzschia* spp. equalled or exceeded the trigger level between 2006 and 2015.

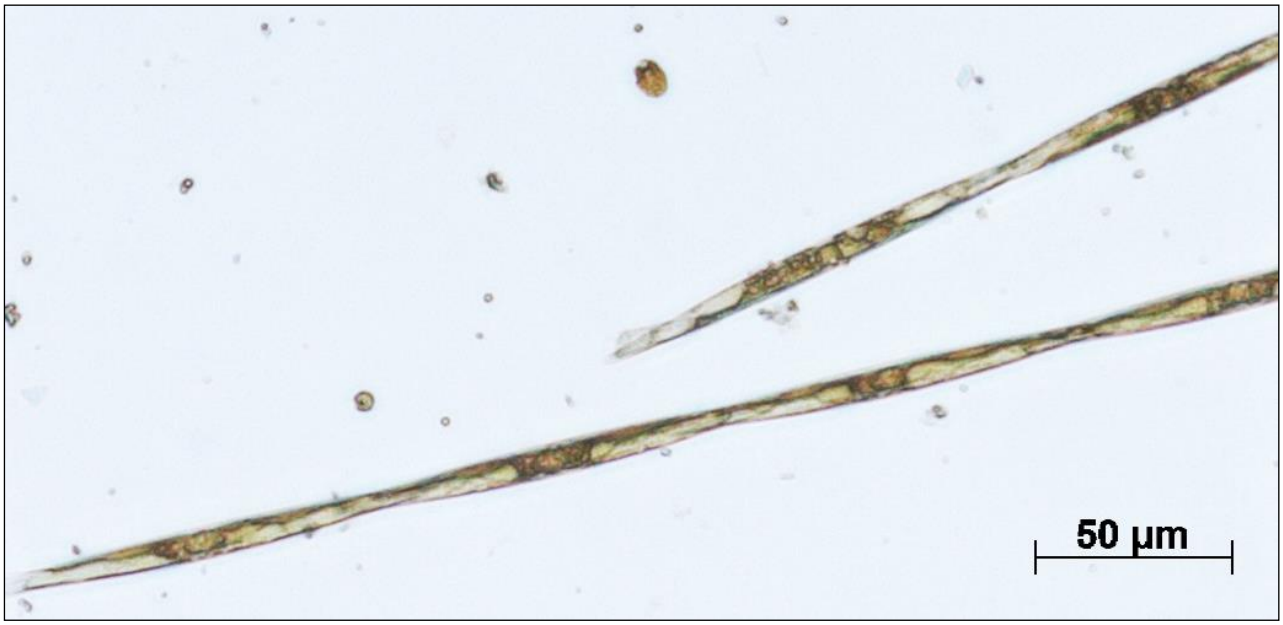
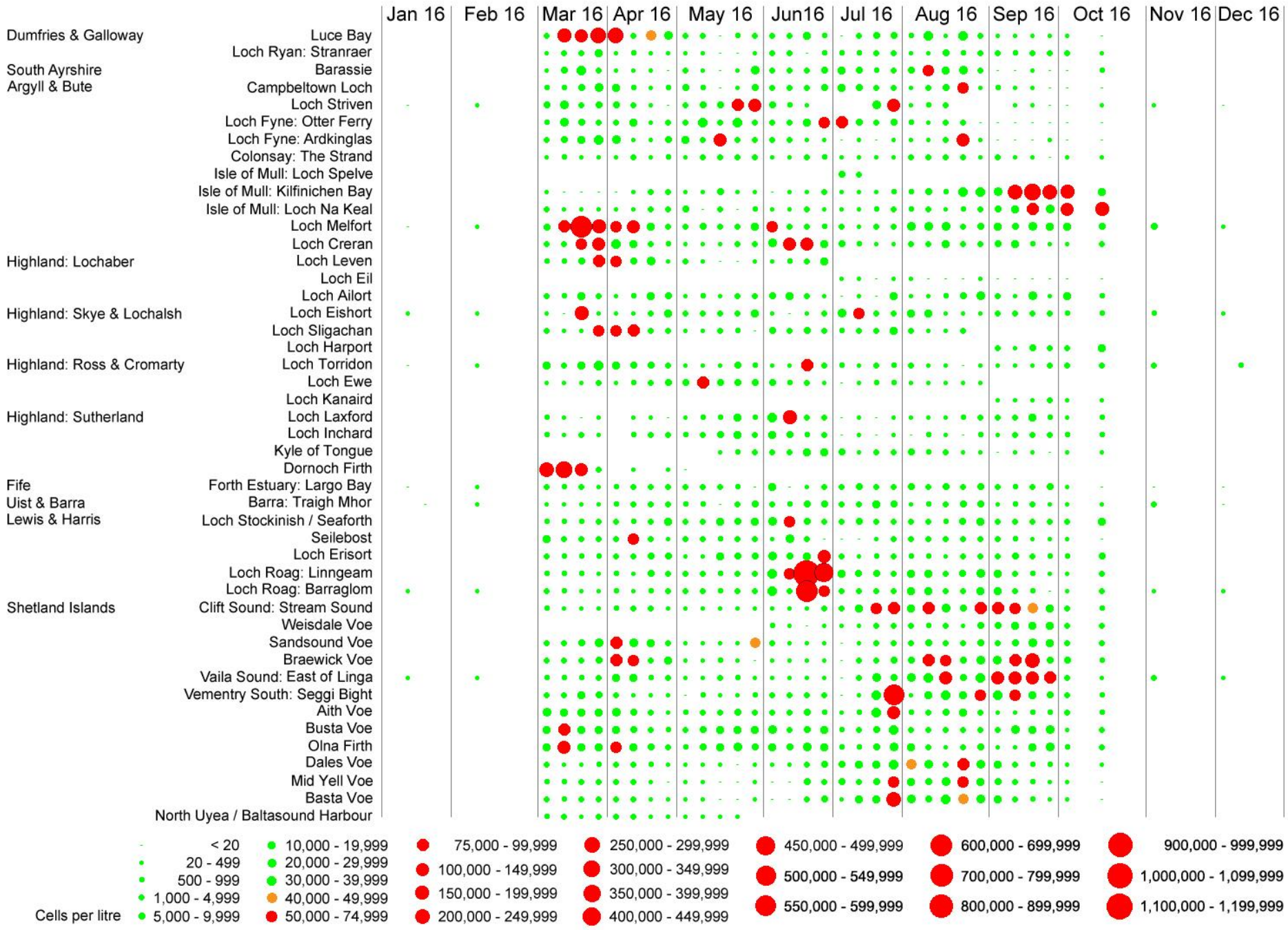


Figure 23: Chains of *Pseudo-nitzschia* spp. observed in Loch Striven (Argyll & Bute) on 1st June.

Figure 24: Phytoplankton concentrations of *Pseudo-nitzschia* spp. observed between January and December 2016



Other potentially harmful phytoplankton

Prorocentrum cordatum was detected in 663 samples analysed in 2016 (50.96%). It was observed from January through to November and was most abundant in May and June, being recorded in 81.72% and 77.19% of the samples analysed, respectively. The densest blooms of 2016 occurred around the Shetland Islands in June, with concentrations of 41,602 cells/L recorded in Weisdale Voe on 27th June, 35,892 cells/L at Clift Sound on 1st June, and 22,906 cells/L in Sandsound Voe on 6th June. It was also relatively abundant around the Highland region (Loch Torridon and Loch Inchard) in June.

Figure 25: *Prorocentrum cordatum* observed in Loch Torridon (Highland: Ross & Cromarty) on 6th June.

The potentially problematic dinoflagellate *Karenia mikimotoi* was observed in densities likely to negatively impact aquaculture during 2016, and was detected in 14.30% of the samples analysed. This species is not an issue in terms of shellfish harvesting, as it does not produce biotoxins that are harmful to human health. However, it does produce ichthyotoxins that can kill finfish, and dense blooms may result in both fish and invertebrate mortality as a result of hypoxia. A dense bloom of *Karenia mikimotoi* was observed around the Firth of Clyde in July and August, with a maximum density in excess of four million cells/L observed in Loch Ryan (Dumfries & Galloway) on 25th July. The bloom persisted through September and into October at this site.

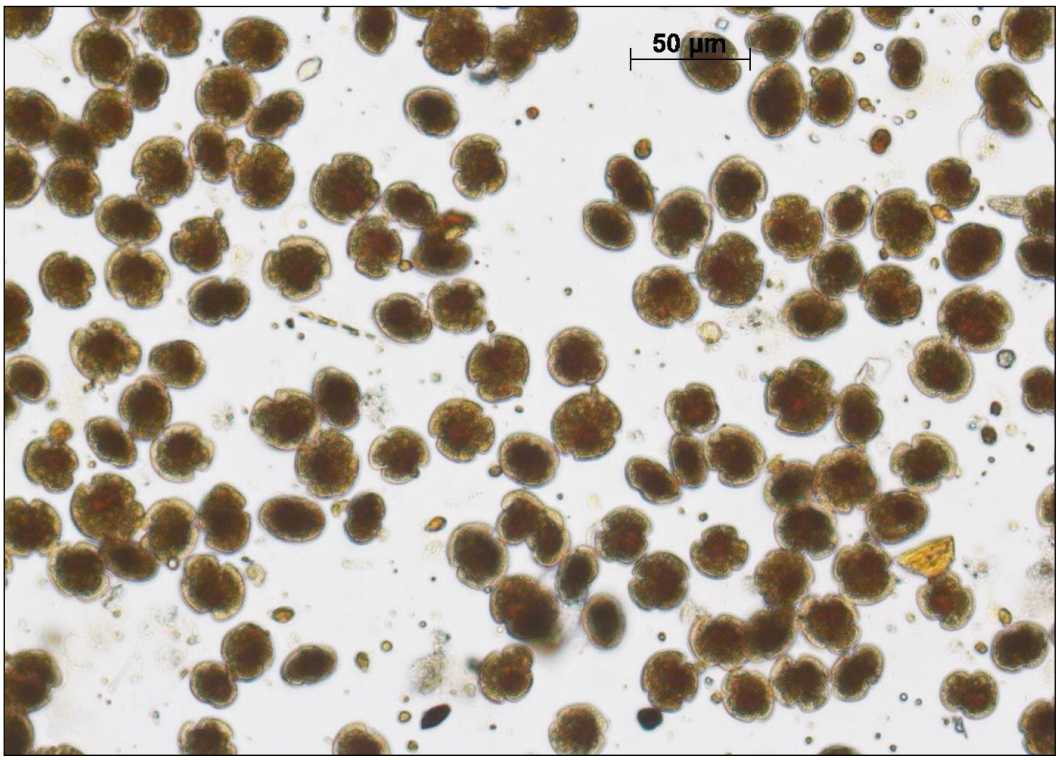


Figure 26: *Karenia mikimotoi* observed in Loch Ryan (Dumfries & Galloway) on 25th July.

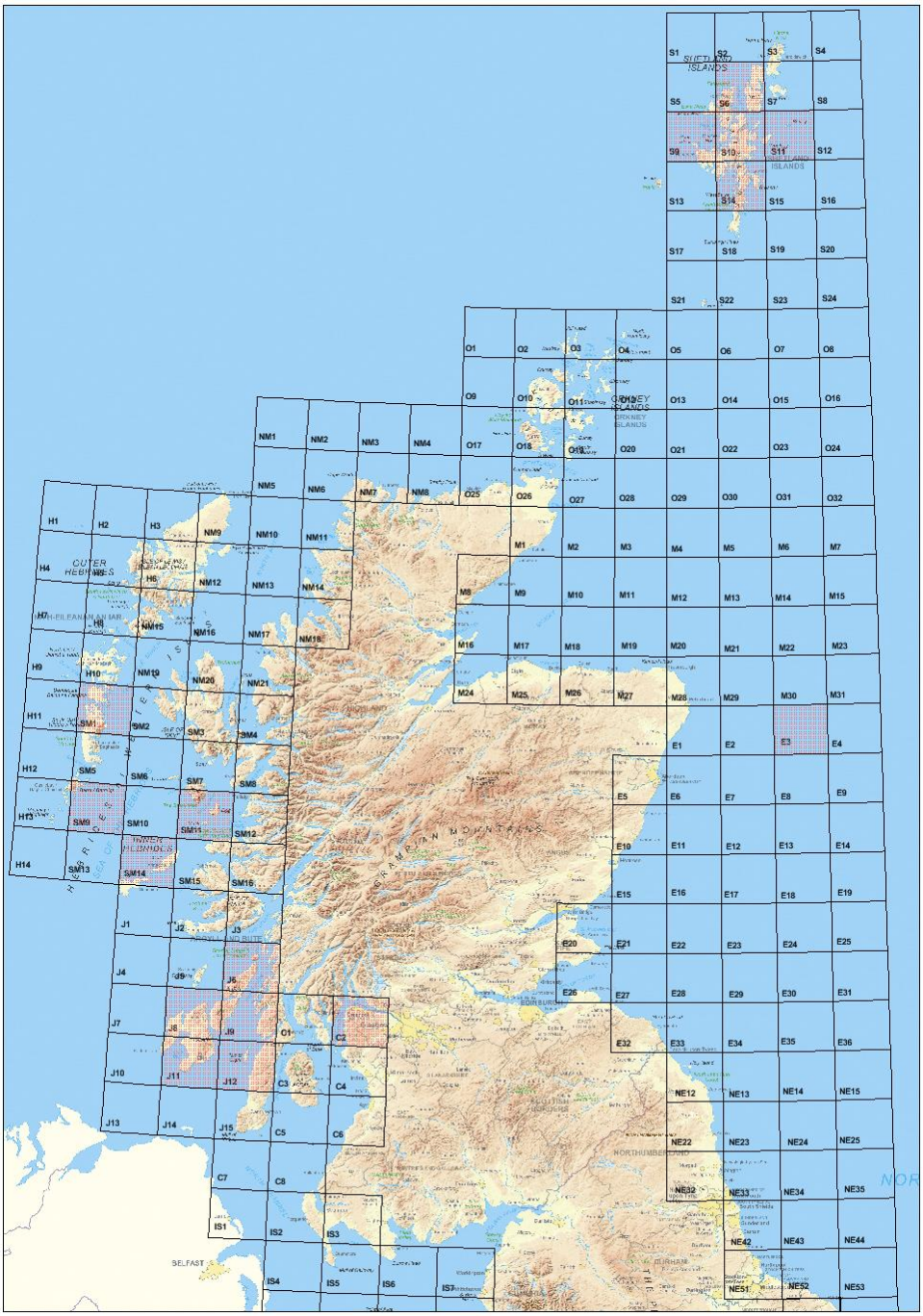
2. Abbreviations used in the text

AHA	Associated Harvesting Area
AOAC	AOAC International
ASP	Amnesic Shellfish Poisoning
AZA	Azaspiracid
AZP	Azaspiracid Poisoning
CI	Cyclic Imines
DA	Domoic Acid
DSP	Diarrhetic Shellfish Poisoning
DTX	Dinophysistoxin
dcSTX	decarbamoyl saxitoxin
EC	European Commission
EU	European Union
EURL	European Union Reference Laboratory for Marine Biotoxins
EHO	Environmental Health Officer
EPT	End product test
FSA	Food Standards Agency
FSS	Food Standards Scotland
GTX	Gonyautoxin
HPLC	High Performance Liquid Chromatography
LA	Local Authority
LC-MS/MS	Liquid Chromatography with tandem Mass Spectrometry
LOD	Limit of detection
LOQ	Limit of quantitation
LT(s)	Lipophilic Toxin(s)
MPL	Maximum Permitted Level
ND	Not Detected
UKNRL	UK National Reference Laboratory for Marine Biotoxins
OA	Okadaic Acid
PSP	Paralytic Shellfish Poisoning
PST(s)	Paralytic Shellfish Toxin(s)
PTX	Pectenotoxin
PTX2	Pectenotoxin 2
PTX2sa	Pectenotoxin 2 seco-acid
RL	Reporting limit
RMP	Representative Monitoring Point
SAMS	The Scottish Association for Marine Science
SOP(s)	Standard Operating Procedure(s)
STX	Saxitoxin
YTX	Yessotoxin

3. Results of the wild pectinidae onshore verification programme

ASP, PSP and LTs analyses were performed on 28 samples from 10 separate establishments received via the wild pectinidae onshore verification programme. The origin of harvest for the scallop samples received during the reporting period (when specified by the sampling officer) is indicated by the shaded cells in Figure 27.

Figure 27: Origins of the wild pectinidae samples received via the FSS onshore official control verification programme in 2016



ASP results

- ASP was detected in 6 king scallop verification samples from 5 establishments. Five of these samples comprised of whole king scallop material, the remaining one of shucked product. These shellfish samples were originally harvested in the following offshore scallop grounds; Jura (4 samples), Clyde (1 samples), South Minch (1 sample). Toxin levels ranged between 1.7 and 39mg/kg DA/shellfish flesh, one of which exceeded the MPL.
- The sample which exceeded the MPL, comprised of whole scallops, originating from the Clyde 02 offshore scallop ground collected by Argyll & Bute Council between in February 2016.

Lipophilic toxin results

- OA/DTX/PTX group toxins below the MPL were detected in one whole king scallop verification sample, from the Clyde 02 scallop ground in February 2016.
- YTX and AZA group toxins were not detected in the any of the 28 samples analysed via the onshore verification programme.

PSP results

- PSP toxins were detected in one sample of whole king scallops, collected from the Clyde scallop ground in February 2016. The sample did not exceed the MPL (395µg STX/kg).

4. Biotoxin Methodology

4.1. Shellfish collection

Inshore Monitoring Programme (classified shellfish production areas):

For the monitoring period of 1st January to 31st December 2016, 2,882 bivalve shellfish samples from 83 inshore sampling locations were submitted for toxin analyses. These sampling locations covered 83 pods within 10 Local Authority regions (13 regional offices).

The inshore samples received by Cefas during the reporting period comprised of mussels (*Mytilus* spp.) (1,875 samples - 65% of all samples), Pacific oysters (*Crassostrea gigas*) (543 - 19%), razors (*Ensis* spp.) (254 – 9%), common cockles (*Cerastoderma edule*) (138 – 5%), surf clams (*Spisula solida*) (42 - <1%), Queen scallops (*Aequipecten opercularis*) (14 - <1%) and carpet clams (*Venerupis senegalensis)* (16 - <1%).

Samples were collected by officers operating on behalf of several contractors appointed by FSS. A list is provided in Table 2. The majority of samples were collected by appointed sampling officers. However, in specific incidences and dependent on location or accessibility, FSS also allowed the collection of samples by the industry. These samples qualified as “unverified” were collected under the direction of the responsible sampling contractor. During this reporting period, 15% of the samples received were of unverified origin. Numbers however, varied significantly between Local Authority regions. A further breakdown of unverified samples received (by species and fishery type) is provided in Table 3.

Table 2: Number of verified and unverified inshore biotoxin samples collected during the reporting period by Local Authority region and by sampling contractor

Local Authority	Sampling contractor	No. samples received	No. verified samples received	No. unverified samples received
Argyll & Bute Council	Argyll & Bute Council	759	732 (96%)	27 (4%)
Comhairle nan Eilean Siar: Lewis & Harris	Hall Mark Meat Hygiene	293	266 (91%)	27 (9%)
Comhairle nan Eilean Siar: Uist & Barra	Hall Mark Meat Hygiene	201	140 (70%)	61 (30%)
Dumfries & Galloway Council	FSS Operations	78	48 (62%)	30 (38%)
Fife Council	Hall Mark Meat Hygiene	91	10 (11%)	81 (89%)
East Lothian Council	Hall Mark Meat Hygiene	41	0	41 (100%)
Highland Council: Lochaber	Highland Council	191	110 (58%)	81 (42%)
Highland Council: Ross & Cromarty	Highland Council	116	116 (100%)	0 (0%)
Highland Council: Skye & Lochalsh	Highland Council	118	117 (99%)	1 (1%)
Highland Council: Sutherland	Highland Council	188	171 (91%)	17 (9%)
North Ayrshire Council	FSA Operations	46	46 (100%)	0 (0%)
Orkney Islands Council	Hall Mark Meat Hygiene	0	0	0
Shetland Islands Council	Hall Mark Meat Hygiene	710	688 (97%)	22 (3%)
South Ayrshire Council	FSS Operations	49	1 (2%)	48 (98%)
Totals		**2,881***	**2,445 (85%)**	**436 (15%)**

*One sample arrived without paperwork and the laboratory was unable to identify the sender.

Table 3: Number of unverified inshore biotoxin samples collected during the reporting period by species and fishery type.

Species	Fishery type	No. of samples received	No. unverified samples received	Proportion of unverified samples received per species
Common cockles	Wild harvest	138	0	0%
Common mussels	Aquaculture	1875	120	6.4%
Common mussels	Wild harvest	0	0	
Pacific oysters	Aquaculture	543	3	<1%
Razors	Wild harvest	254	248	98%
Surf clams	Wild harvest	42	40	95%
Carpet clams	Wild harvest	16	16	100%
Queen scallops	Wild harvest	14	9	64%

Shellfish were collected and packaged in accordance with the Shellfish Partnership sampling and transport protocol, itself based upon UKNRL guidance and sent to the Cefas Weymouth laboratory for analyses. All samples were posted using Royal Mail next day delivery service. The majority of samples (~99%) arrived at the laboratory within one or two working days of sample collection (78 and 21%, respectively) (Table 4). When delays occurred, these were generally attributed to the time at which the samples were collected, thus missing the routine post office collection deadline or to other events outside of the laboratory or sampling officers' control, such as inclement weather or transport network problems.

Table 4: Number of inshore samples received from each Local Authority region and time taken between collection and receipt at Cefas in 2016

Local Authority	No. samples received	No. received 1 working day post collection	No. received 2 working days post collection	No. received 3 working days post collection	No. received 4 working days post collection
Argyll & Bute Council	759	648	104	7	0
Comhairle nan Eilean Siar: Lewis & Harris	293	251	42	0	0
Comhairle nan Eilean Siar: Uist & Barra	201	109	84	7	1
Dumfries & Galloway Council	78	64	13	1	0
Fife Council	91	45	46	0	0
East Lothian Council	41	14	27	0	0
Highland Council: Lochaber	191	105	81	5	0
Highland Council: Ross & Cromarty	116	102	11	3	0
Highland Council: Skye & Lochalsh	118	82	34	2	0
Highland Council: Sutherland	188	170	18	0	0
North Ayrshire Council	46	42	4	0	0
Orkney Islands Council	0	0	0	0	0
Shetland Islands Council	710	584	118	8	0
South Ayrshire Council	49	24	25	0	0
Totals	**2,881***	**2,240 (78%)**	**607 (21%)**	**33 (1%)**	**1 (<1%)**

*One sample bag arrived with no paperwork and the source could not be identified. This sample was not tested

Careful programme management, training and liaison with sampling officers minimised the occurrence and impact of delays on the programme, with only ~1% of samples (n=33) being received three or four working days post collection throughout this reporting period. Only one of these late samples was rejected as unsuitable for analyses, based on poor organoleptic properties upon arrival at the laboratory (see section 4.2).

Wild pectinidae – Onshore Surveillance Programme:

Twenty-eight king scallop samples (comprising of shucked product (n=24) or whole shellfish (n=4)) were collected from 12 separate premises by authorised officers from three LA regions (Argyll & Bute, Comhairle nan Eilean Siar: Uist & Barra and Shetland Isles) during the reporting period and submitted to Cefas for toxin analyses.

The scallop samples were originally harvested from the following offshore scallop grounds: Clyde (C02), East (03), Jura (J06, J08, J09, J11 & J12), Shetlands (S06, S09, S10, S11 & S14) and South Minch (SM01, SM09, SM11 & SM14) (Figure 27, page 39).

Twenty-four samples arrived within one working day of collection, with four samples received two working days post collection.

4.2 Shellfish analysis

Assessment of suitability of the samples for analysis

On arrival at the laboratory, all samples were assigned a unique laboratory number and assessed for their suitability for analysis.

Shellfish which failed to respond to a percussion test, and/or did not exhibit the correct organoleptic characteristics associated with freshness or were accompanied by incorrect or missing paperwork were rejected and reported as unsuitable for analyses. A summary of the number of samples assessed as unsuitable during the reporting period is given in Table 5. Overall, only twelve inshore samples were rejected in 2016. No king scallop verification samples were rejected as unsuitable for analysis. Therefore 99.5% of all samples received were assessed as suitable for analysis and tested in 2016.

Table 5: Summary of inshore samples found unsuitable for toxin analyses, by Local Authority region.

Local Authority	No. samples received	No. rejected due to unsatisfactory quality or provenance	No. rejected due to other reasons (e.g.: arrived late or unscheduled sample)
Argyll & Bute Council	759	1	0
Comhairle nan Eilean Siar: Lewis & Harris	293	0	0
Comhairle nan Eilean Siar: Uist & Barra	201	2	0
Dumfries & Galloway Council	78	1	1
Fife Council	91	0	0
East Lothian Council	41	0	0
Highland Council: Lochaber	191	0	0
Highland Council: Ross & Cromarty	116	0	0
Highland Council: Skye & Lochalsh	118	0	2
Highland Council: Sutherland	188	1	0
North Ayrshire Council	46	0	1
Orkney Islands Council	0	0	0
Shetland Islands Council	710	0	0
South Ayrshire Council	49	0	3
Totals	**2,882***	**6* (0.21 %)**	**7 (0.24%)**

*One sample bag arrived with no paperwork and the source could not be identified. This sample was not tested

Insufficient samples

Samples which were assessed as suitable for analysis were then prepared for ASP, LTs and/or PSP analyses (as required). The analyses to be conducted on each batch of samples were defined by the current risk assessment and co-ordinated by Cefas. All inshore and king scallop verification samples assessed as suitable for analyses yielded sufficient material for the required tests.

C. Methodology of shellfish analysis

The methods used for routine toxin analysis of shellfish were those specified by the FSA and involved the application of a range of analytical methods. These included liquid chromatography (LC) with Ultra-violet (UV) or fluorescence (FLD) detection or LC with tandem mass spectrometry (MS/MS) for either qualitative screening of samples (screen), semi-quantitation or full toxin quantitation. The methods used for toxin testing were as follows:

ASP testing

- Shellfish species received in the reporting period were tested by LC-UV analysis following extraction with 50% aqueous methanol and filtration of the crude extracts. The quantitative method was applied to all shellfish species and is based on the method of Quilliam et al., 1995.
- ASP results are reported as mg/kg of domoic and epi-domoic acid combined

PSP testing

- Shellfish species received in the reporting period have all been validated at Cefas for the use of a refined LC-FLD method based on AOAC 2005.06. Samples were all extracted with 1% acetic acid and forwarded for qualitative screening and semi-quantitation by LC-FLD. Any samples returning a positive LC screen result and a semi-quantitative total toxicity of >400 µg STX eq/kg were then forwarded for quantitation by LC-FLD.
- Screen positive samples under this limit were reported as <400 µg STX eq/kg. Since implementation, this approach has significantly increased the number of sample results reported within 1 day of sample receipt and increased the ability of the laboratory to deal with large numbers of positive samples during periods of high PSP toxicity.
- Quantitation was conducted following the fully quantitative AOAC 2005.06 method, with final results reported as total toxicities in µg STX eq/kg.

Lipophilic toxins testing

- All shellfish species were analysed by LC-MS/MS for the quantitation of all EU regulated lipophilic toxins. The method used was validated at Cefas based on the conditions stipulated by the EU Reference Laboratory (EU RL) for Marine Biotoxins.
- Results are reported as total toxicities in µg eq/kg for the OA, AZA and YTX groups separately.

Appended table 2 summarises the methods of analysis used throughout this reporting period together with a summary of the current UKAS accreditation status of each method to ISO 17025:2005 standard.

Table 6: List of analytical methods used, by species, in 2016

Toxin group	Methods employed	Species tested	Dates	Accreditation status (as of 31st December 2016,) to ISO 17025:2005 standard
ASP	LC-UV	All species	1st January to 31st December 2016	Accredited
PSP	LC-FLD (screen, semi-quantitative screen & full quantitation)	All species	1st January to 31st December 2016	Accredited
Lipophilic toxins	LC-MS/MS	All species	1st January to 31st December 2016	Accredited

Table 7: Flesh and phytoplankton trigger levels

Toxin group	Levels of toxin or cell concentrations triggering additional monitoring if breached
ASP	≥10mg domoic/epi-domoic acid/kg shellfish flesh and/or *Pseudo-nitzschia* spp. ≥ 50,000 cells/L
LTs	OA/DTX/PTX group: ≥80 µg OA eq/kg shellfish flesh AZA group: ≥80 µg AZA1eq./kg shellfish flesh YTX group: ≥1.8mg/kg shellfish flesh and/or *Prorocentrum lima*/*Dinophysis* spp. ≥ 100 cells/L
PSP	≥400µg STX eq./kg shellfish flesh and/or *Alexandrium* spp. (40 cells/L)

5.2 Reporting of results

Upon completion of the required analyses, the results were collated and quality control checked prior to submission to FSS.

Results were reported on a daily basis. During this reporting period, Cefas were able to report individual results from 99% of all tests carried out within one working day of receipt and 100% within two working days (Table 8).

Of the 51 samples results which were reported after one working day of receipt, 30 samples (59%) required additional PSP LC-FLD quantitative analyses, thus incurring a delay in the reporting timeframe.

For reference, the turnaround times agreed with FSS and required from Cefas during the reporting period were as follows:

Table 8: Sample turnaround times (from sample receipt) specified by FSS and achieved by the laboratory

Toxin and analysis method	FSA specified targets	Laboratory statistics in the reporting period (all results combined)
ASP by HPLC	80% within 1 working day 100% within 3 working days	99% within 1 working day 100% within 2 working days
Lipophilic toxins by LC-MS	70% within 1 working day 100% within 3 working days	
PSP by HPLC (screen)	80% within 1 working day 100% within 3 working days	
PSP by HPLC (quantitation)	80% within 2 working days 100% within 4 working days	

Required turnaround times were therefore all met and for all analyses, delivery by the laboratory exceeded the targets agreed with FSS.

In addition to daily reports, all results from samples received between Monday and Friday the previous week were collated and reported in a weekly results sheet to FSS, released by the following Tuesday.

A summary of results turnaround times, for inshore samples from day of receipt to completion of all required analyses for the period 1st January to 31st December 2016 is given in Table 9.

Table 9: Turnaround times, by Local Authority region, for samples received from inshore areas in 2016

Local Authority	No. samples received	No. completed results reported within one working day of receipt of sample	No. completed results reported two working days after receipt of sample	No. completed results reported three working days after receipt of sample
Argyll & Bute Council	759	739	14 20	0
Comhairle nan Eilean Siar: Lewis & Harris	293	288	5	0
Comhairle nan Eilean Siar: Uist & Barra	201	196	5	0
Dumfries & Galloway Council	78	77	1	0
Fife Council	91	91	0	0
East Lothian Council	41	41	0	0
Highland Council: Lochaber	191	191	0	0
Highland Council: Ross & Cromarty	116	113	3	0
Highland Council: Skye & Lochalsh	118	115	3	0
Highland Council: Sutherland	188	181	7	0
North Ayrshire Council	46	44	2	0
Orkney Islands Council	0	0	0	0
Shetland Islands Council	710	707	3	0
South Ayrshire Council	49	47	2	0
Totals	**2,881***	**2,830 (98%)**	**51 (2%)**	**0 (0%)**

*One sample bag arrived with no paperwork and the source could not be identified. This sample was not tested

5 Phytoplankton Methodology

5.1 Water collection

For the monitoring period 1st January to 31st December 2016, a total of 1,305 seawater samples were collected from 53 sampling locations within 7 Local Authority regions (11 local offices) (Table 10). As for shellfish samples, seawater samples were collected by officers operating on behalf of several contractors appointed by the FSS. A list is provided in Table 10.

Table 10: Number of water samples collected during the reporting period by Local Authority region and by sampling contractor.

Local Authority	Sampling contractor	No. samples received	No. samples rejected
Argyll & Bute Council	Argyll & Bute Council	294	2
Comhairle nan Eilean Siar: Lewis & Harris	Hall Mark Meat Hygiene	165	1
Comhairle nan Eilean Siar: Uist & Barra	Hall Mark Meat Hygiene	36	0
Dumfries & Galloway Council	FSS Operations	64	0
Fife Council	Hall Mark Meat Hygiene	37	1
Highland Council: Lochaber	Highland Council	64	0
Highland Council: Ross & Cromarty	Highland Council	68	0
Highland Council: Skye & Lochalsh	Highland Council	67	0
Highland Council: Sutherland	Highland Council	91	0
Shetland Islands Council	Hall Mark Meat Hygiene	387	0
South Ayrshire Council	FSS Operations	32	0
TOTALS		**1,305**	**4**

Samples were collected and packaged in accordance with SRSL's guidance and protocols and sent to the SRSL Oban laboratory for analysis. Three samples were not analysed as they were not required, due to the reduced autumn sampling schedule. One sample was rejected because it had been collected from a site not on the current phytoplankton RMP list. This resulted in a total of 1,301 samples being analysed between 1st January and 31st December 2016.

The sampling protocol used by appointed officers followed that described by the UKNRL SOP for the collection of water samples for toxic phytoplankton analysis. The aim of this method is to collect samples of phytoplankton that are representative of the community in the water body. The water sample is taken as close to the shellfish bed as possible and at the same location from where shellfish samples for tissue analysis are collected. The sampling method used depends on the depth of water at the site, and water samples are collected with either a PVC sample tube (the preferred method) or a bucket, as appropriate. A well-mixed 500 mL sub-sample of this water is then preserved using Lugol's iodine and returned (usually by post) to SRSL for analysis.

The majority of samples (99.08%) arrived at the laboratory within one or two working days of sample collection, 89.35% and 9.73%, respectively (Table 11). Of the samples taking

more than one working day to arrive, 82.01% were from remote areas, with the majority of these samples being collected on islands (78.07%).

Table 11: Number of phytoplankton samples received from each Local Authority region and time taken between collection and receipt at SRSL in 2016.

Local Authority	No. samples received	No. received 1 working day post collection	No. received 2 working days post collection	No. received 3 working days post collection	No. received ≥4 working days post collection
Argyll & Bute Council	294	264	26	3	1
Comhairle nan Eilean Siar: Lewis & Harris	165	148	17	0	0
Comhairle nan Eilean Siar: Uist & Barra	36	24	11	1	0
Dumfries & Galloway Council	64	62	2	0	0
Fife Council	37	23	13	1	0
Highland Council: Lochaber	64	44	19	1	0
Highland Council: Ross & Cromarty	68	59	9	0	0
Highland Council: Skye & Lochalsh	67	52	14	1	0
Highland Council: Sutherland	91	91	0	0	0
Shetland Islands Council	387	370	13	3	1
South Ayrshire Council	32	29	3	0	0
TOTAL SAMPLES	**1,305**	**1,166**	**127**	**10**	**2**
Percentage of total		**89.35%**	**9.73%**	**0.77%**	**0.15%**

Phytoplankton analysis

Assessment of suitability of the samples for analysis

On arrival at the laboratory, all samples were assigned a unique laboratory number and assessed for their suitability for analysis.

Methodology

The UKNRL protocol for the identification and enumeration of potential toxin-producing phytoplankton was used to analyse all water samples. In the laboratory, a sub-sample of 50 mL is routinely settled (Figure 28), but if the amount of sediment present in the sub-sample is excessive, 25 mL or 10 mL sub-samples may be used.

Figure 28: Phytoplankton cells in a 50 mL sub sample of Lugol's-fixed seawater are allowed to settle onto the base plate of the chamber prior to analysis.

The phytoplankton cells within the sub-sample are allowed to sink onto the base of a settling chamber for a minimum period of 20 hours (for a 50 mL sub-sample) before analysis. The cells are then identified and enumerated using an inverted light microscope. Final cell densities are calculated to express phytoplankton concentration as the number of cells per litre (cells/L) of sample. The method is accredited to ISO 17025 standard.

Test outcome

"Trigger" levels for toxic phytoplankton concentrations in the water column have been determined historically by comparing phytoplankton count data with the presence of biotoxins in shellfish tissue. Trigger levels remained at the same cell concentrations as used in 2015 (Table 7, Page 46).

5.2 Reporting of results

Upon completion of analyses, results were collated and quality control checked prior to submission to the FSS. During 2016, SRSL was able to report all results within three working days of sample receipt. This turnaround time is in full compliance with the targets specified by the FSS (98% of results reported within 3 working days of sample receipt).

In addition to the daily reporting schedule, all results from samples received the previous week were collated and reported in a weekly results sheet to FSS, released by the following Tuesday.

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