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Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for Scotland - 2017

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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 or SRSL.

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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 2017.

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 2,161 bivalve shellfish samples from 88 inshore sampling locations (Figure 1) were submitted to Cefas for toxin analyses in the reporting period. They comprised of; common mussels (1,525), Pacific oysters (399), razors (142), common cockles (60), surf clams (31), and native oysters (4).

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

Six inshore samples (<0.3% of those received) were rejected on arrival at the laboratory – three of these were submitted in error as testing was not required in these areas, the remaining three arrived at the laboratory in a condition unsuitable for analyses (1 sample contained undersized shellfish and two razors samples contained less than 10 individual shellfish).

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

Phytoplankton monitoring

A total of 1,352 seawater samples from 45 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 1,351 were analysed. One sample was not analysed due to the reduced autumn sampling schedule.

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

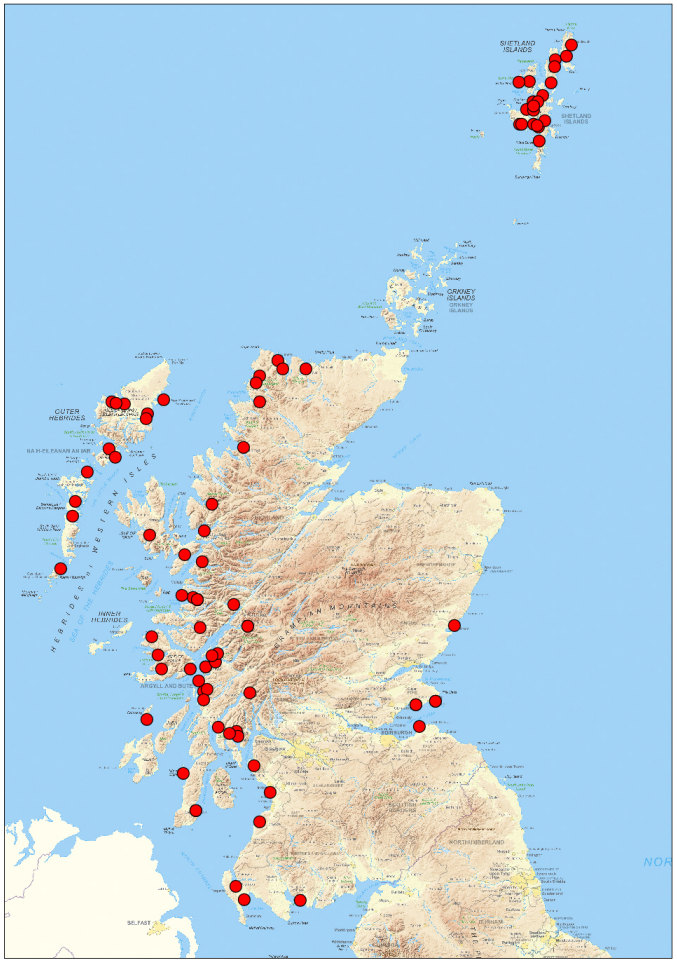


Figure 1: Scottish inshore shellfish sampling locations – Food Standards Scotland biotoxin monitoring programme in 2017

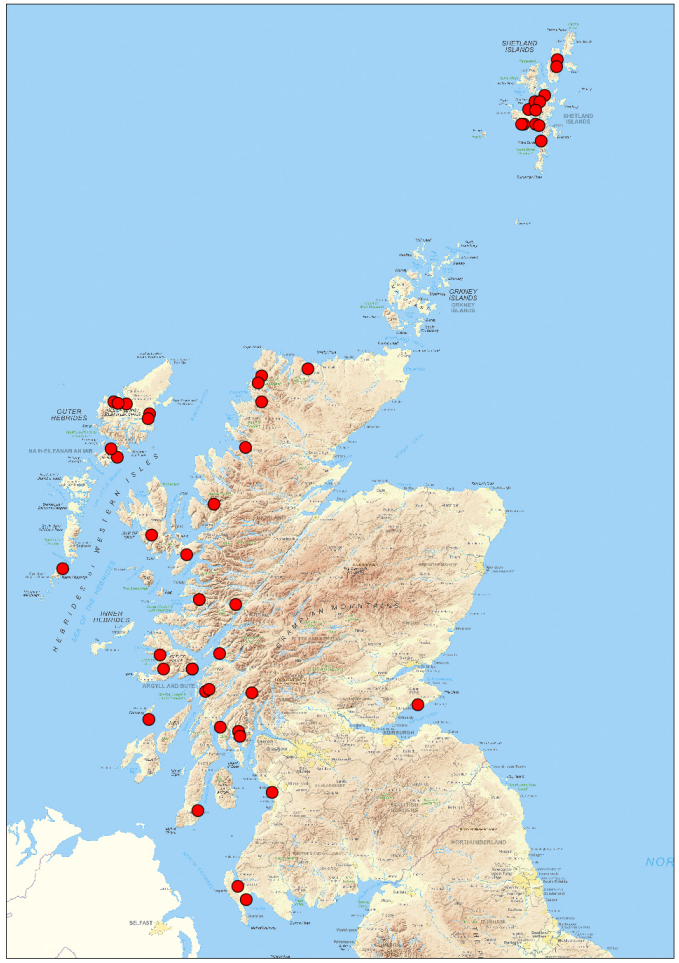


Figure 2: Scottish water sampling locations – Food Standards Scotland phytoplankton monitoring programme in 2017

2. 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.

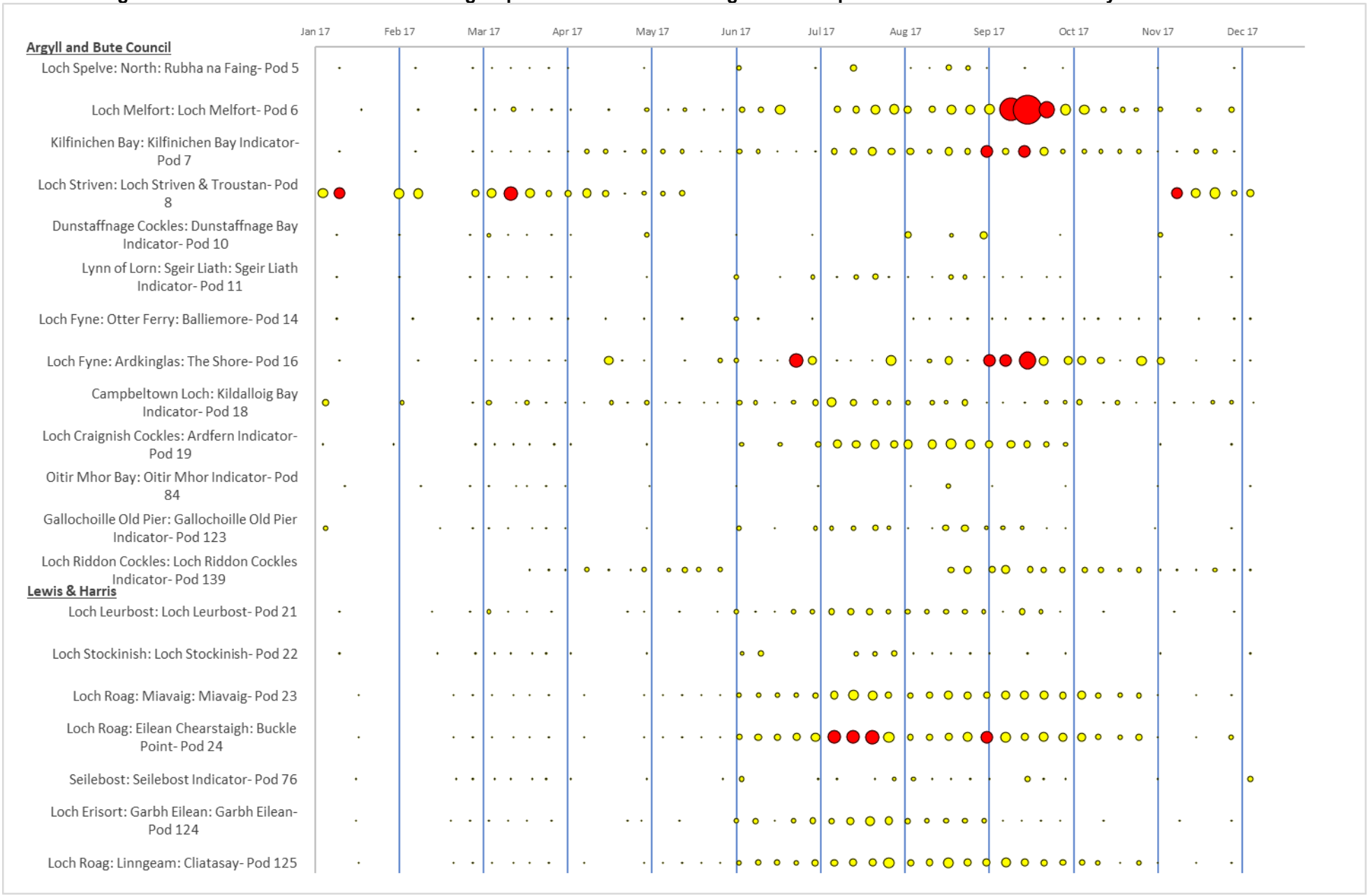
During this reporting period, 46 inshore samples breached maximum permitted levels (MPL) for lipophilic toxins. This is the lowest number of breaches since the LC-MS/MS method was introduced in 2011. As observed previously, where monitoring for lipophilic toxins had occurred in the previous two to three 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 2,056 samples from inshore locations and 15 verification samples collected from commercial establishments. Results are summarised below.

2.1 OA/DTX/PTX group

- OA/DTX/PTX group toxins were detected in 730 inshore samples, comprising of mussels (700 samples), Pacific oysters (23) and surf clams (7).
- 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 2017 (636 samples).
- The distribution of OA/DTX/PTX toxins was widespread, affecting sites within all monitored local authority regions, with the exception of Comhairle nan Eilean Siar – Uist and Barra.
- Forty six samples comprising of mussels (40 samples) and Pacific oysters (6) from 15 sites recorded results above the MPL. Results above MPL were predominantly recorded between June and November 2017. Two results occurred between Janaury and March 2017 at one site (Loch Striven) (Figure 4). Overall, this is the lowest number of regulatory exceedances since the LC-MS/MS method was introduced in 2011. This is reflected in the algal results for 2017 (see p. 16).
- The highest level recorded during 2016 was 1,054µg OA eq./kg, more than six times the regulatory limit, in a sample from Loch Laxford (Highland: Sutherland) in mid July 2017. Levels of OA/DTX/PTX group toxins at this site had fluctuated during the weeks prior to this peek, rising from 34µg OA eq./kg to 432µg OA eq./kg within the space of one week in late May/early June, at the commencement of this toxic event.
- Elsewhere, OA/DTX/PTX group toxins were detected below the MPL in a further 684 samples from 58 sites (Figure 5), between January and December 2017. This level of detection is comparable to previous years.
- No OA/DTX/PTX group toxins were detected in the king scallop verification samples received in 2017.

Figure 3. Concentrations of OA/DTX/PTX group toxins in sites recording results at quantifiable levels from January to December 2017



Concentration of OA/DTX/PTX group toxins:

2,000 µg OA eq/kg

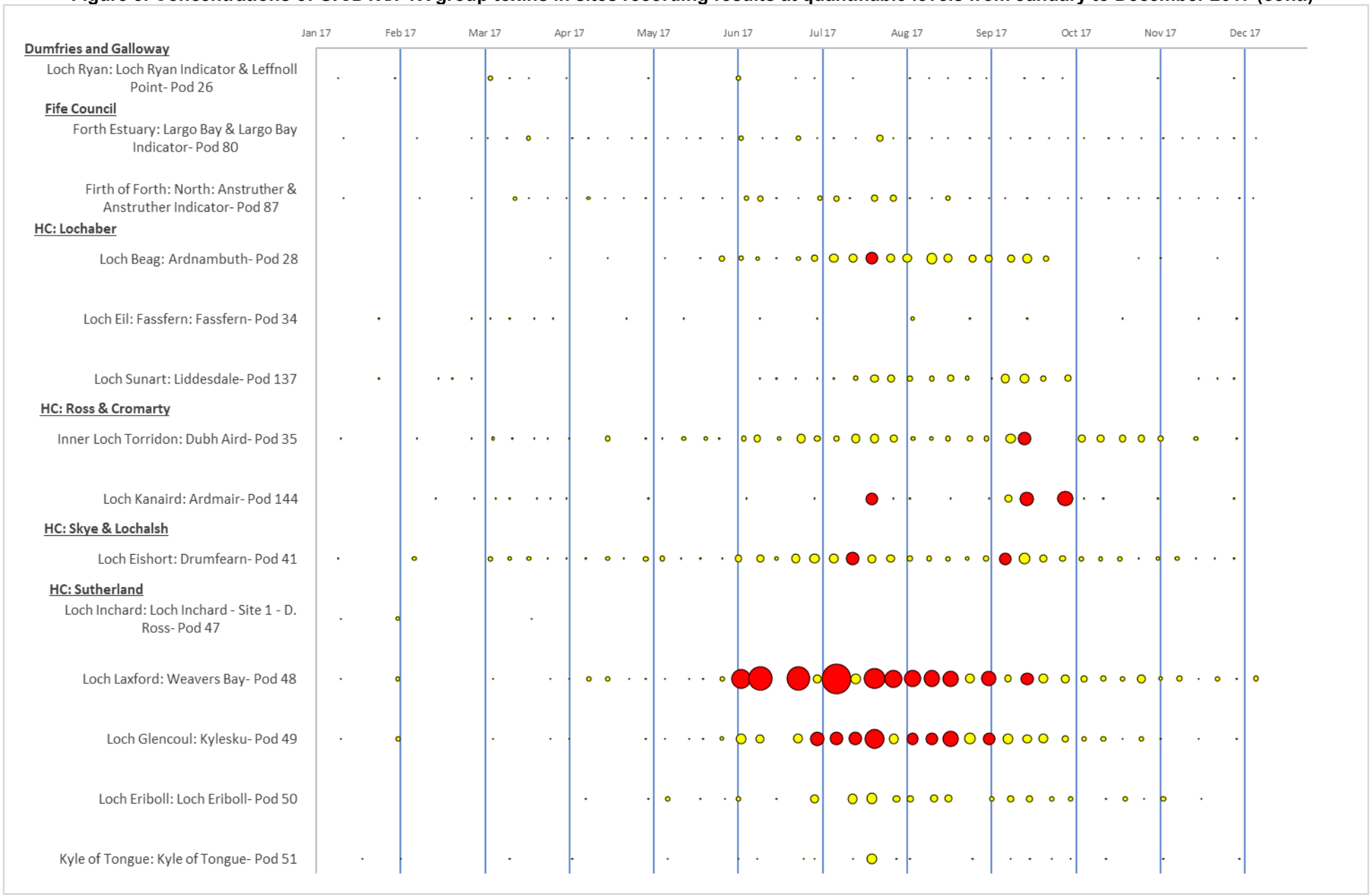
1,000 µg OA eq/kg

>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 in sites recording results at quantifiable levels from January to December 2017 (cont.)



Concentration of OA/DTX/PTX group toxins:

2,000 µg OA eq/kg

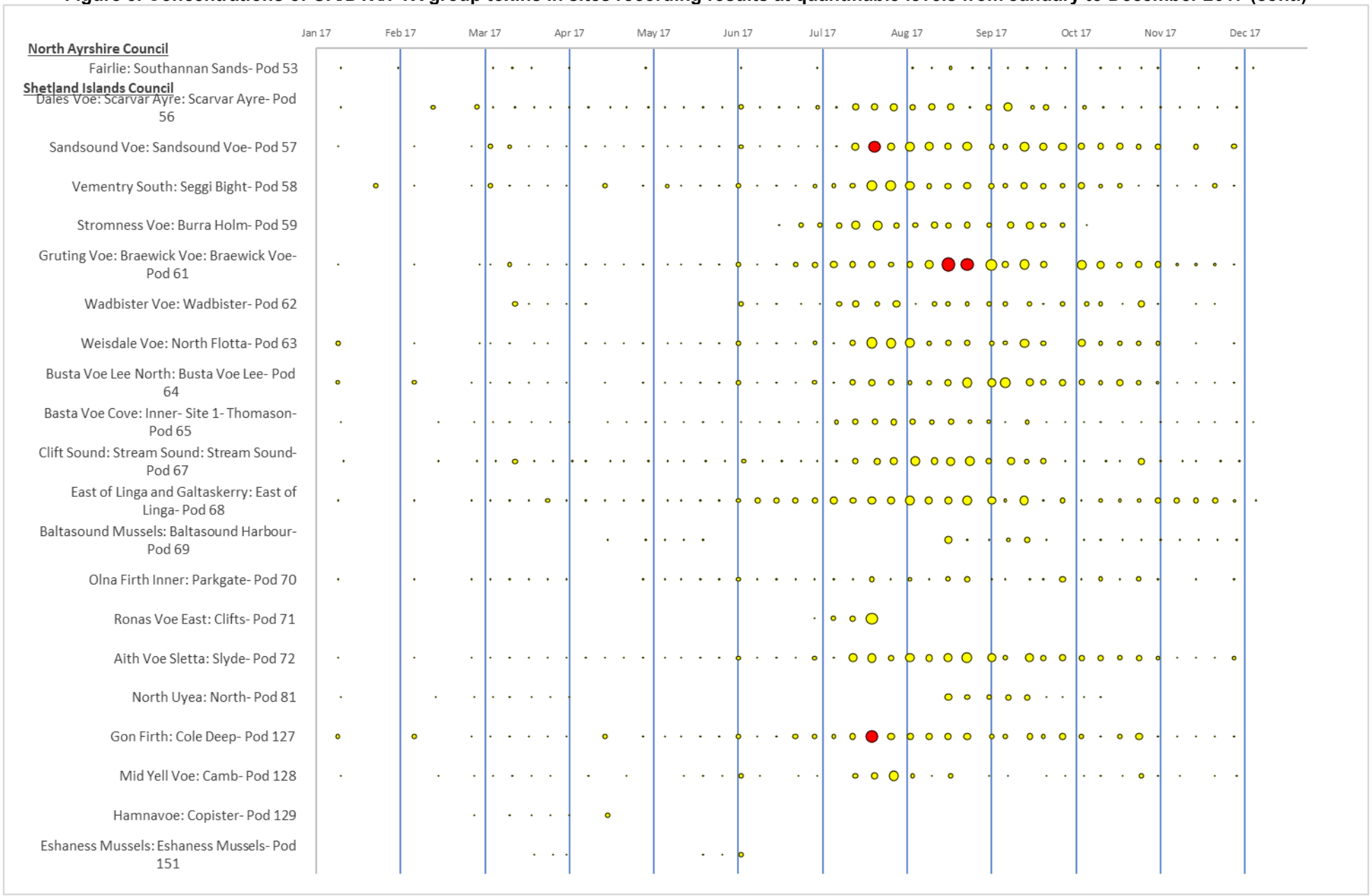
1,000 µg OA eq/kg

>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 in sites recording results at quantifiable levels from January to December 2017 (cont.)



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

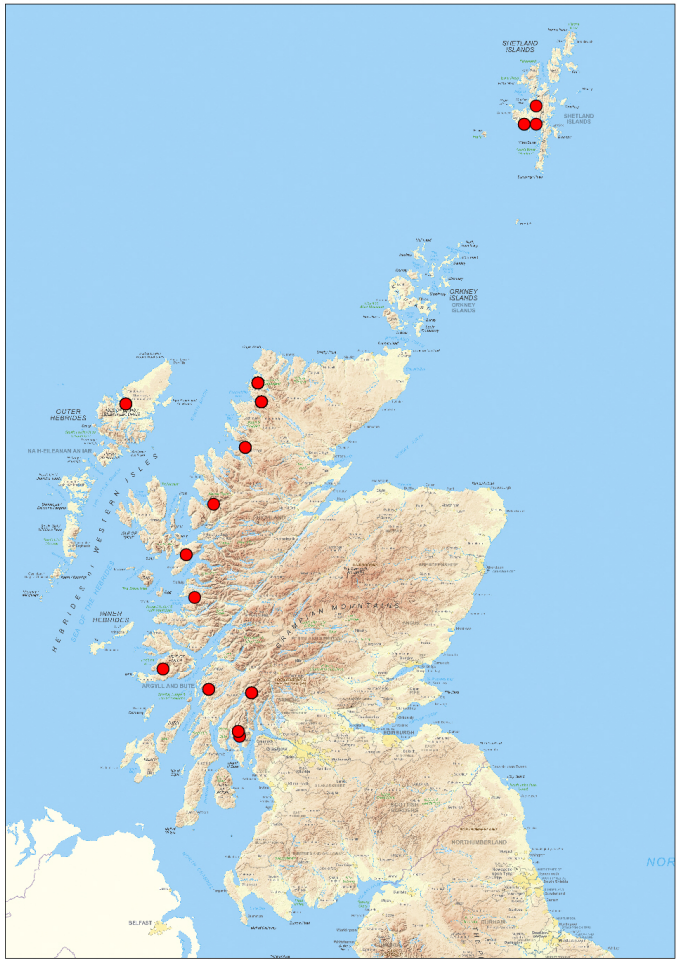


Figure 4: Inshore locations recording OA/DTX/PTX group results above the maximum permitted limit (>160µg OA eq./kg) in 2017

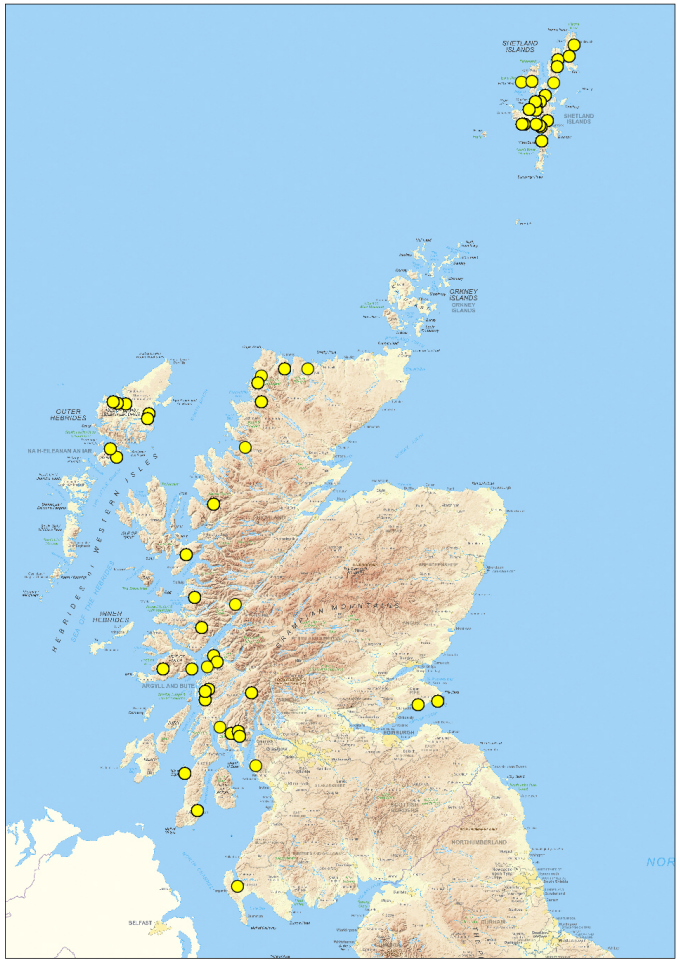


Figure 5: Inshore locations where toxins of OA/DTX/PTX group were detected below the maximum permitted limit (≤160µg OA eq./kg) in 2017

2.2 AZA group

- AZAs were detected in three samples in 2017. Two were collected from Pod 12 - Loch A Chumhainn at 21 & 22µg AZA eq./kg, in late March/early April and one sample collected from Pod 4 - Seil Point: Poll' a Bhrochain (Cyster) at 23µg AZA eq./kg also in late March (Figure 6). All three samples consisted of Pacific oysters, no additional samples were required.

2.3 YTX group

- YTXs were not detected during the reported period.

Figure 6: Inshore locations where AZA group toxins were detected in 2017 (all below the maximum permitted level (≤160µg AZA eq./kg))

2.4 Phytoplankton associated with the production of lipophilic toxins

- *Dinophysis* spp.* were present in 511 (37.8%) of the 1,351 samples analysed during 2017 and were detected in January, and from March to October (Figure 8). They were observed at or above trigger level (set at 100 cells/L) in 186 samples (13.8%) between March and October. The majority of *Dinophysis* spp. blooms occurred around the Scottish coast in June, July and August, with 33.0% of the samples exceeding threshold counts in July (Figure 7). The percentage of samples with *Dinophysis* spp. counts above trigger level has peaked during July in ten of the last twelve years of monitoring.
- The earliest bloom** exceeding trigger level was recorded in Loch Fyne: Ardkinglas (Argyll & Bute) on 21st March. Dense blooms of *Dinophysis* spp. were frequently recorded at Loch Fyne: Ardkinglas (Figure 9) between late June and late September, with the highest cell densities reaching 180,289 cells/L on 29th August and 8,600 cells/L on 20th September. These blooms appeared to be confined to upper Loch Fyne, with samples obtained from lower Loch Fyne (Otter Ferry) during the same time period containing *Dinophysis* spp. at concentrations below threshold.
- *Dinophysis* spp. blooms were widespread around the Highland region from mid May into August, with cell counts exceeding 1,000 cells/L in Loch Laxford and Kyle of Tongue (Highland: Sutherland), Loch Harport (Highland: Skye & Lochalsh) and Loch Kanaird (Highland: Ross & Cromarty). Blooms were also recorded around the Shetland Islands, mainly between June and August.
- Overall, the total percentage of *Dinophysis* spp. blooms at or exceeding trigger level during the current reporting period (13.8%) was lower than that in the preceding three years (19.3% in 2014, 19.3% in 2015, and 19.8% in 2016).

*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.

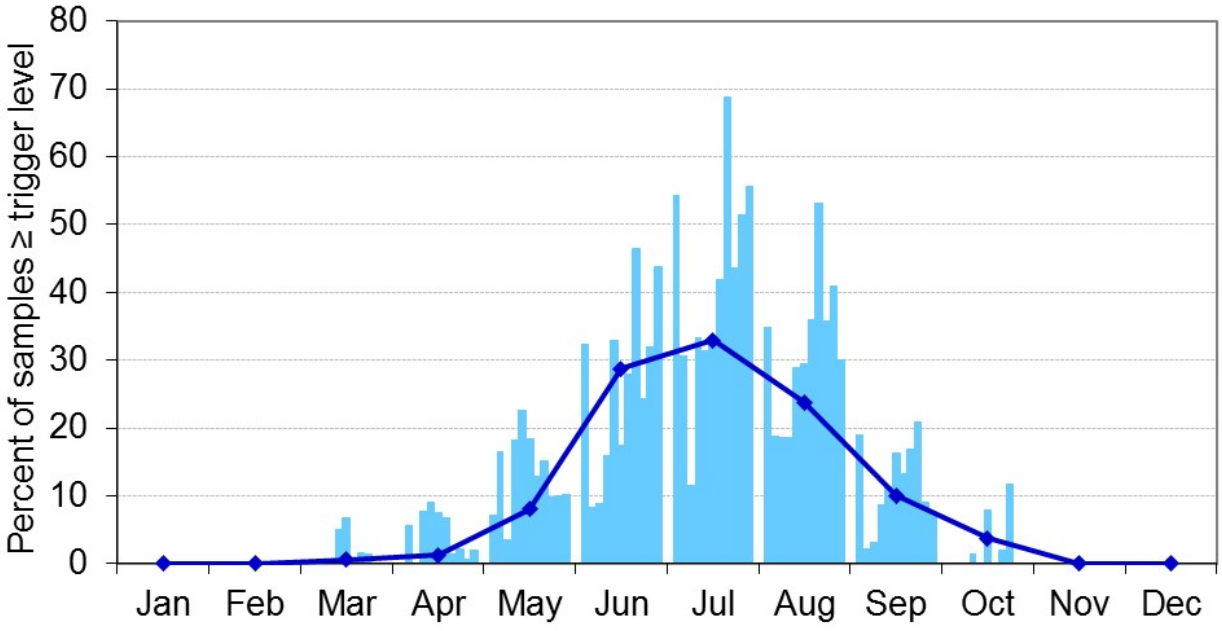
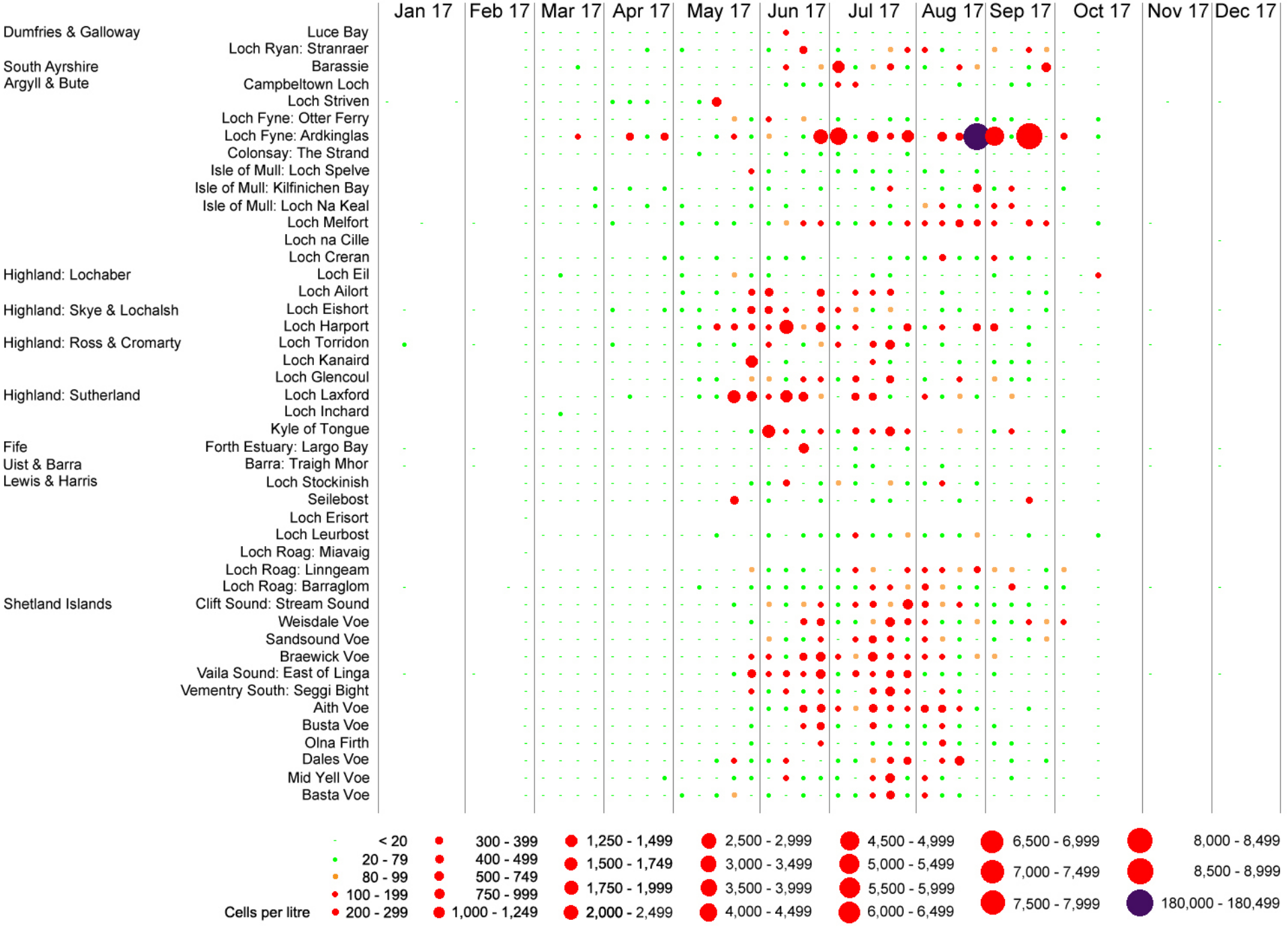


Figure 7: The percentage of samples in which *Dinophysis* spp. equalled or exceeded the trigger level of 100 cells/L in 2017 is indicated by the line. (For comparison, the bars show the percentage of samples in which *Dinophysis* cells equalled or exceeded the trigger level between 2006 and 2016).

Figure 8. Phytoplankton concentrations of *Dinophysis* spp. observed between January and December 2017



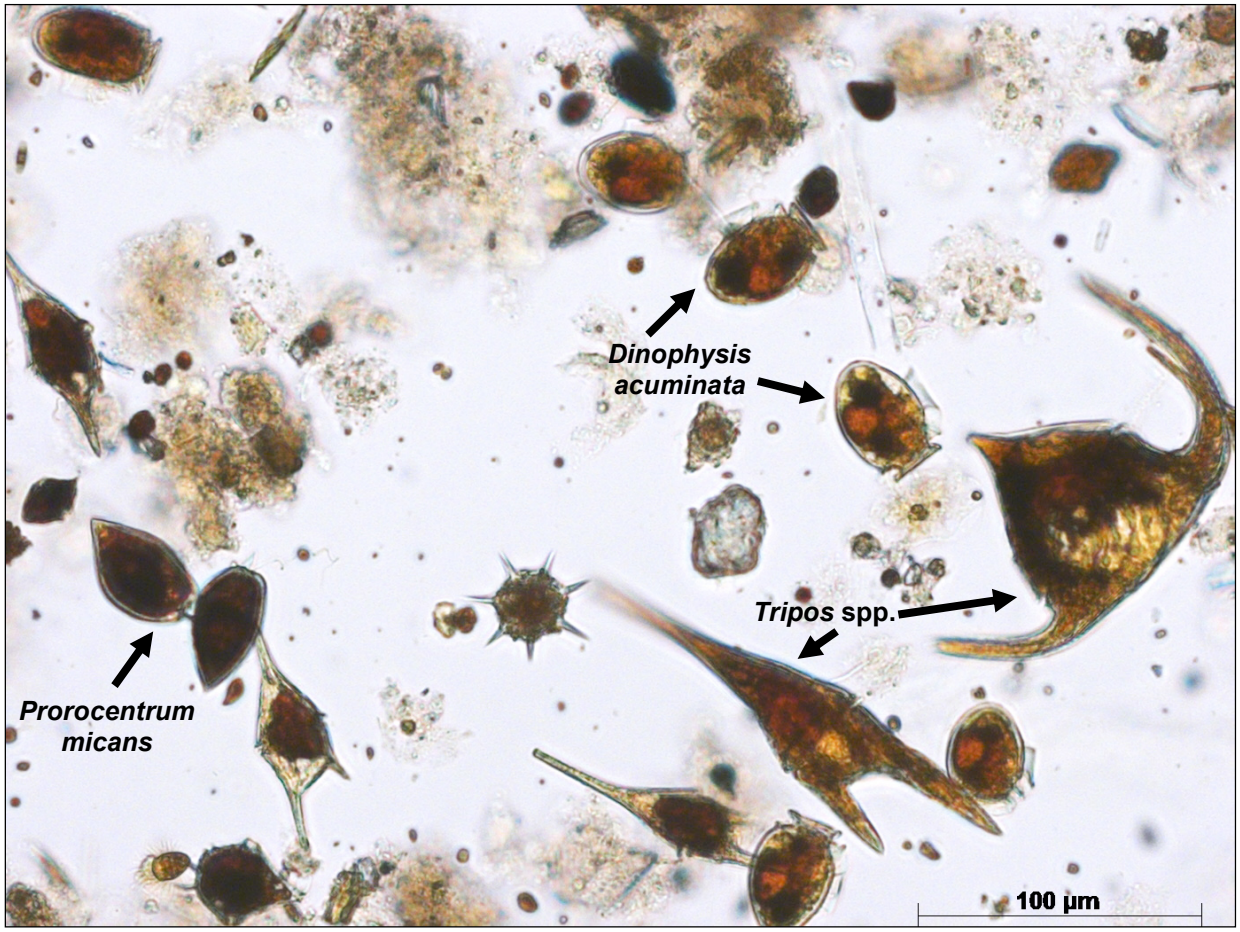


Figure 9: *Dinophysis acuminata* observed on 29th August in Loch Fyne: Ardkinglas (Argyll & Bute). The phytoplankton community was dominated by dinoflagellates including *Prorocentrum micans* and *Tripos* spp.

- The benthic dinoflagellate *Prorocentrum lima* (Figure 10) was present in 248 samples (18.4%) analysed during 2017. It was recorded from February to October, and was reported at or above the trigger level (set at 100 cells/L) in 28 samples (2.1%) collected between April and September. The densest blooms observed in 2017 were both 620 cells/L, recorded at Campbeltown Loch (Argyll & Bute) on 12th June, and in Loch Torridon (Highland: Ross & Cromarty) on 8th August.

- The dinoflagellate *Protoceratium reticulatum* (Figure 11) was not particularly abundant in 2017 and was detected in only 14 samples (1.0%) between April and August. It was most frequently observed around Argyll & Bute, particularly in Loch Fyne, Loch Melfort and on the west coast of the Isle of Mull. The densest bloom occurred in Argyll & Bute, with 140 cells/L recorded in Loch Melfort on 2nd August. No trigger level has been set for *Protoceratium reticulatum*.

- The dinoflagellate *Lingulodinium polyedrum* (Figure 12) is rarely abundant in Scottish coastal waters but was detected on eleven occasions (0.8 % of samples), all from Argyll & Bute and South Ayrshire during 2017. Nine observations were reported between May and September in Loch Creran, where it appears to bloom annually. It was also recorded in Loch Spelve (Argyll & Bute) in September, and at Barassie (South Ayrshire) in August. The maximum bloom density of 180 cells/L

was observed in Loch Creran on 18th September. No trigger level has been set for *Lingulodinium polyedrum*.

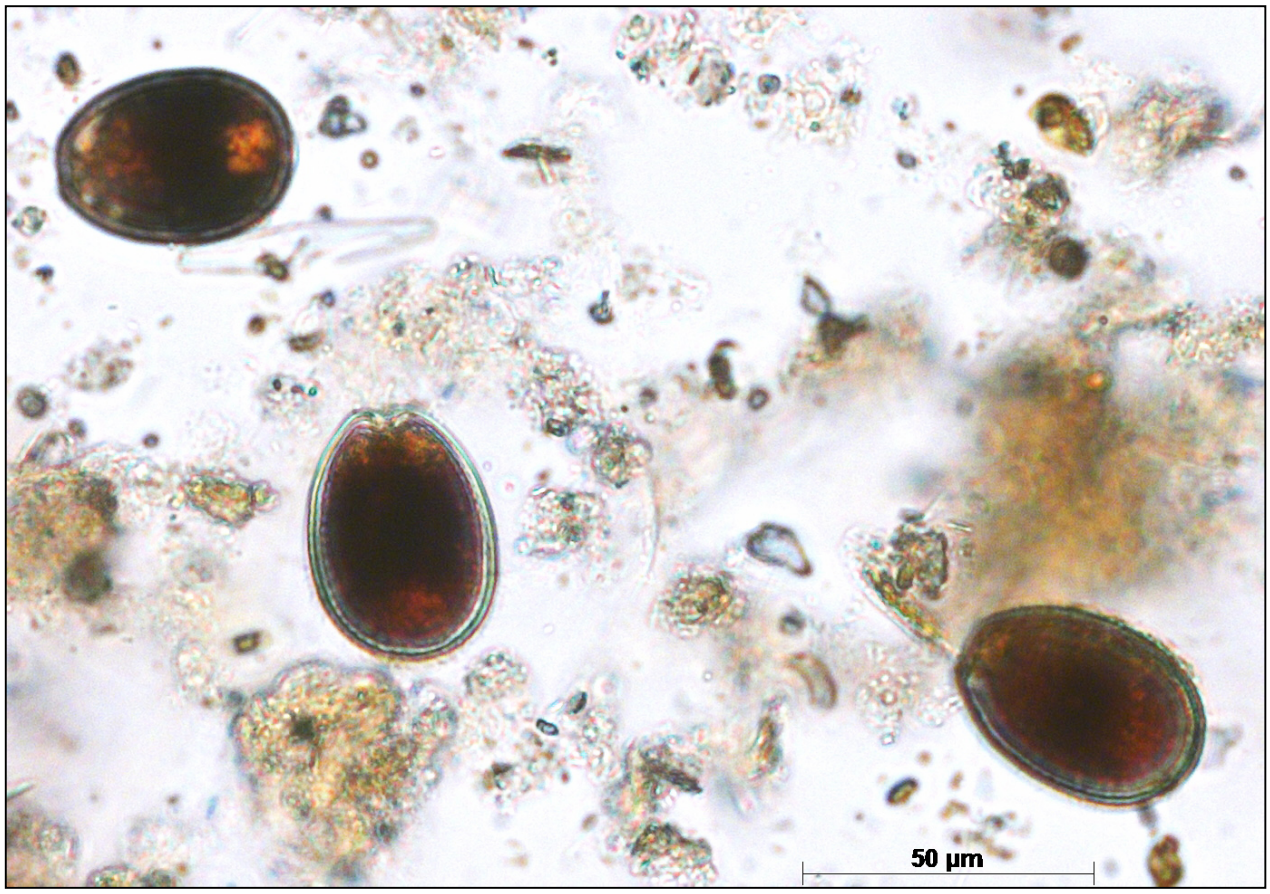


Figure 10: *Prorocentrum lima* observed at Loch Torridon (Highland: Ross & Cromarty) on 8th August.

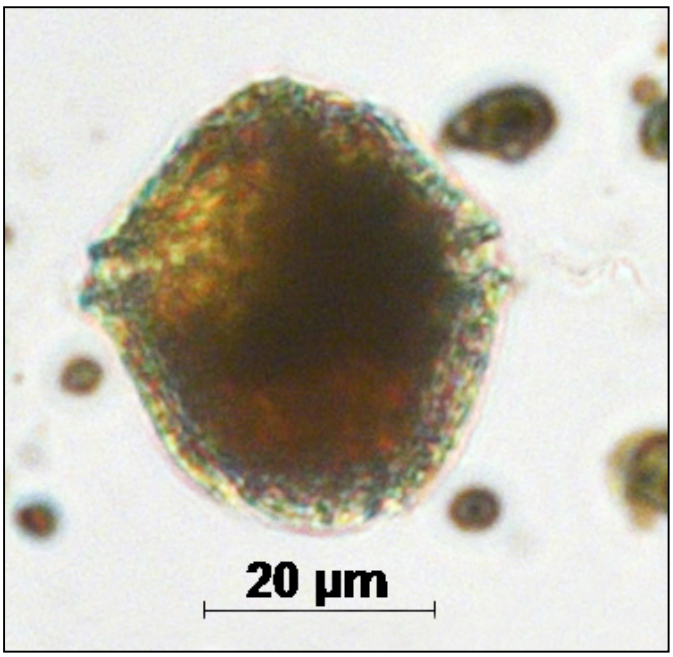


Figure 11: *Protoceratium reticulatum* from Loch Fyne: Ardkinglas (Argyll & Bute) on 1st August.

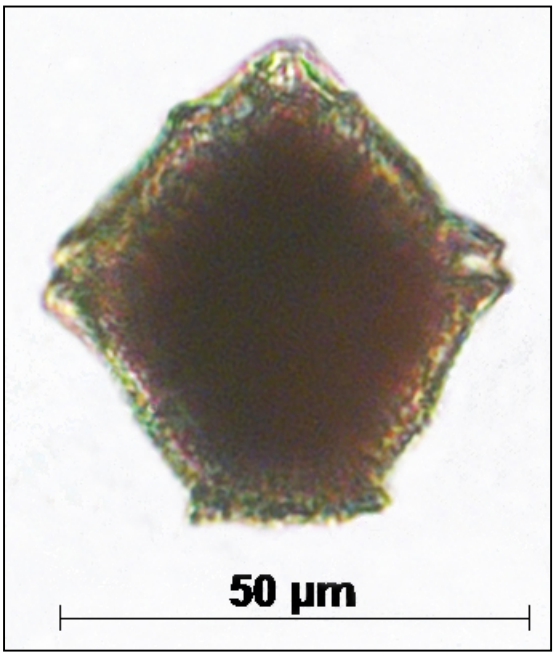


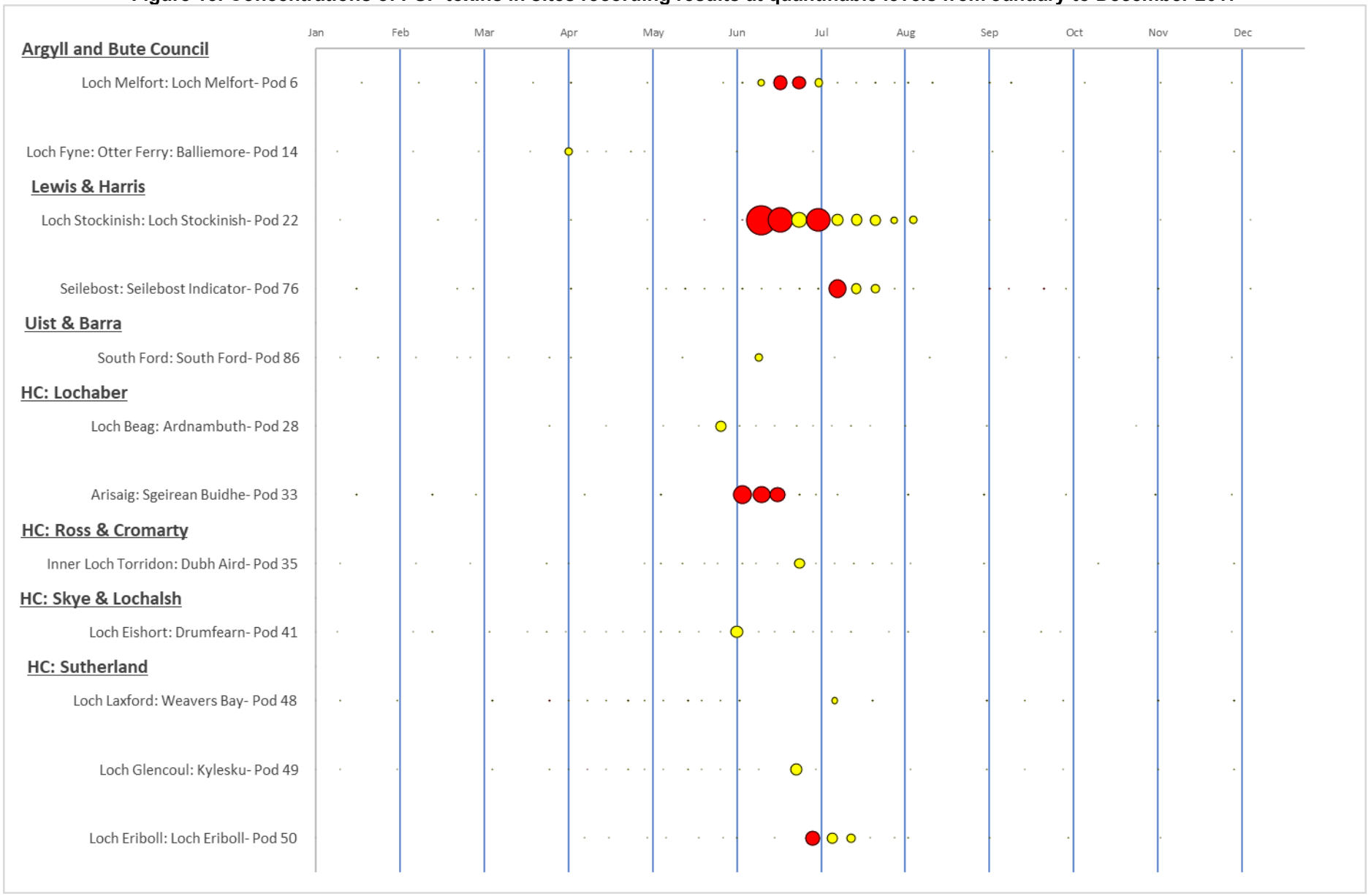
Figure 12: *Lingulodinium polyedrum* from Loch Creran (Argyll & Bute) on 19th July.

3. Monitoring for PSP toxins

A total of 1,380 samples from inshore locations and 15 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.

- Eleven samples from five sites, consisting of mussel (8 samples) and Pacific oysters (3), were found to contain PSP toxins above the MPL of 800µg STX eq./kg shellfish flesh between June and mid July 2017 (Figure 14). The highest level recorded was 4,450µg/kg, over five times the regulatory limit in a mussel sample from Loch Stockinish (Lewis and Harris) collected in early June 2017.
- PSP toxins above reporting levels, but below the MPL were detected in a further 20 samples comprising of mussels (16 samples), Pacific oysters (3) and cockles (1) from 12 sites (Figure 15). All occurrences were recorded between April and August 2017 (Figure 13).
- A range of PSP toxins were quantified during 2017, 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 some 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., with profiles dominated by GTX1&4, GTX2&3 and STX. The majority of samples quantified occurred during June and July and were predominantly mussels, although several pacific oyster samples were also subjected to a quantitation test, along with one cockle sample.
- No quantifiable levels of PSP toxins were detected in the king scallop verification samples.

Figure 13. Concentrations of PSP toxins in sites recording results at quantifiable levels from January to December 2017



Concentration of PSP toxins:

1,000µg STX eq/kg 800µg STX eq/kg (MPL) • Not Detected

Red = Toxins above MPL
Yellow = Toxins below MPL

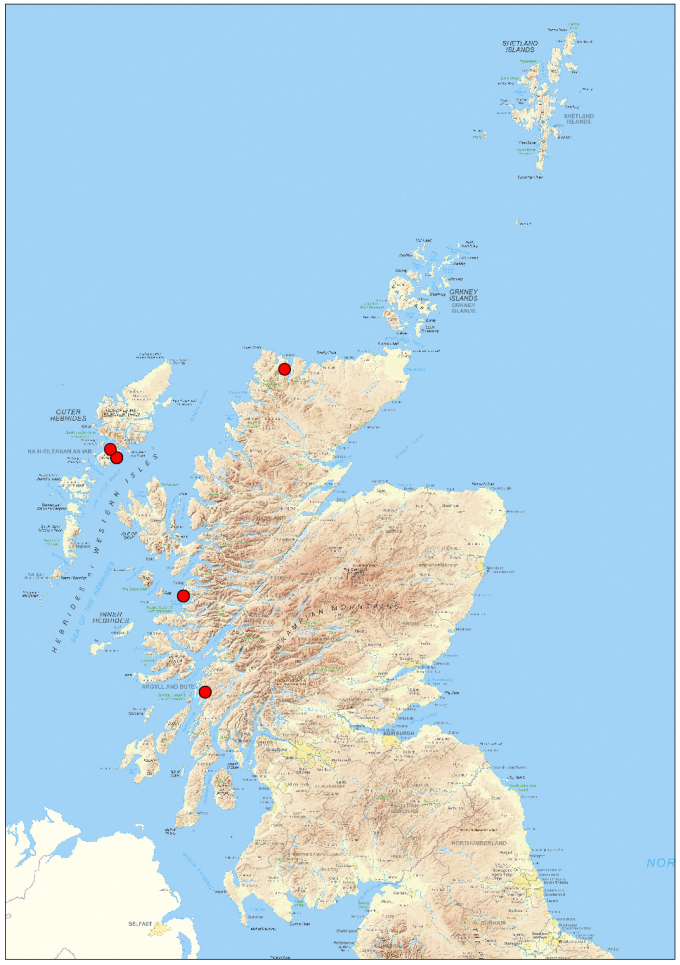


Figure 14: Inshore locations recording PSP toxin results above the maximum permitted limit (>800µg STX eq./kg) in 2017

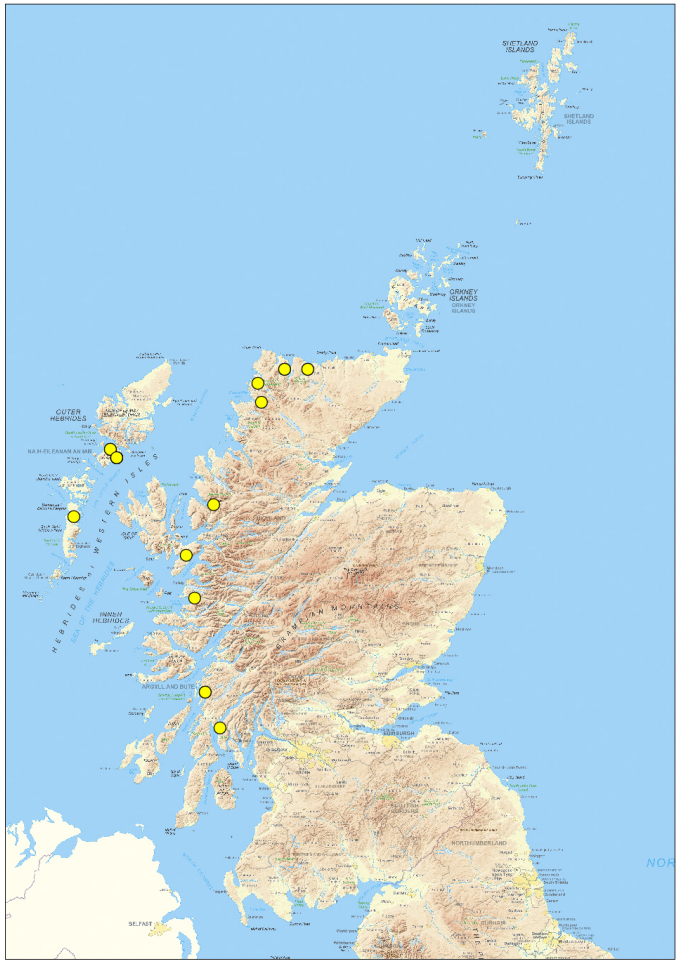
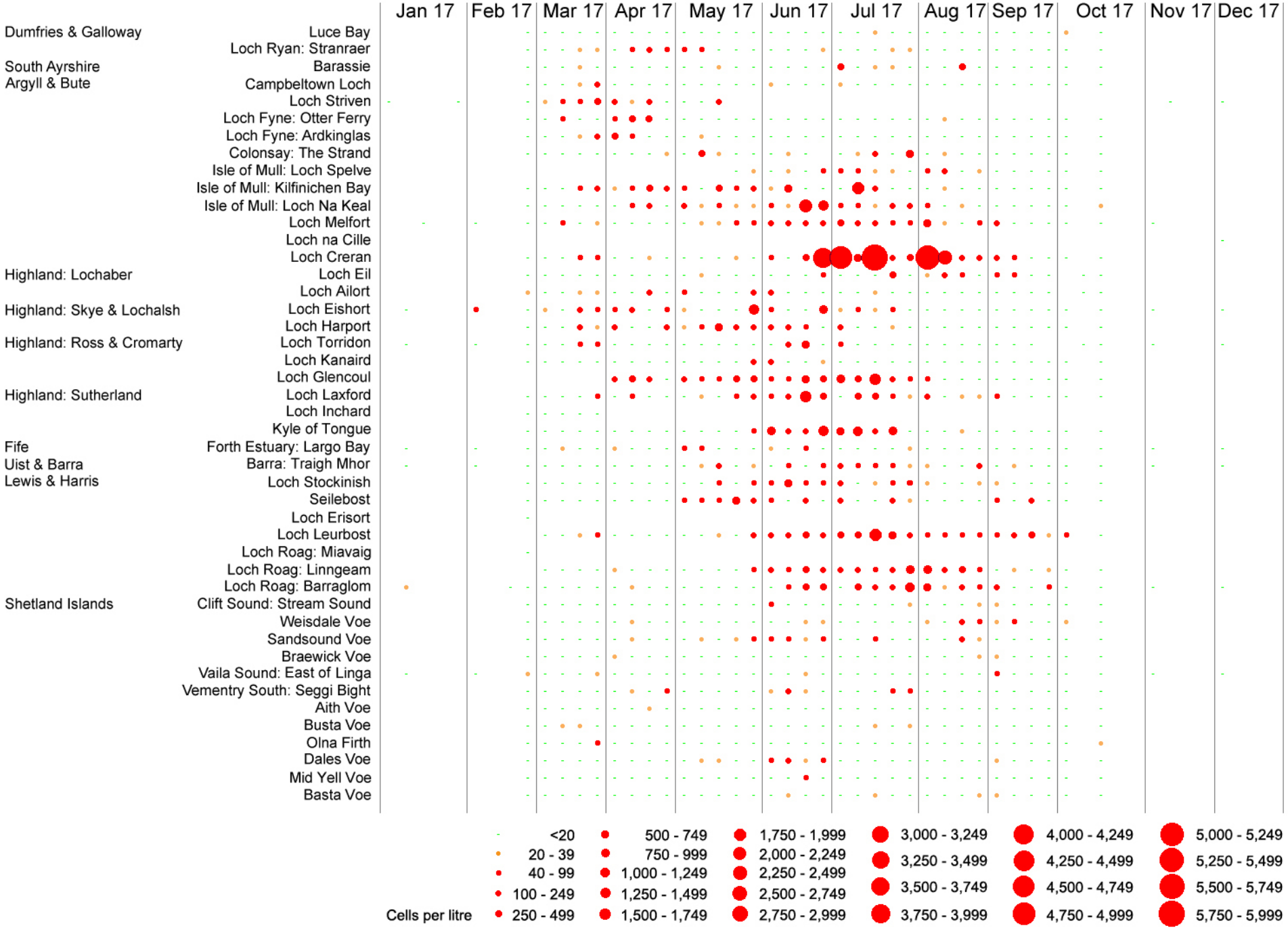


Figure 15: Inshore locations recording PSP toxin results below the maximum permitted limit (≤800µg STX eq./kg) in 2017

3.1 Phytoplankton associated with the production of PSP toxins

- Dinoflagellates belonging to the genus *Alexandrium* were observed between January and October (Figure 16) and were detected in 384 (28.4%) of the 1,351 samples analysed during 2017. They were reported at or above the trigger level (set at 40 cells/L) in 260 samples (19.3%), mostly between June and July. 40% of the samples analysed from June were recorded at or exceeded the trigger level (Figure 17).
- The earliest *Alexandrium* spp. bloom of 2017 that breached trigger level was recorded in Loch Eishort (Highland: Skye & Lochalsh) on 6th February. Blooms were detected in other areas around Argyll & Bute and in the Highland region during spring (March and April), but cell counts remained relatively low and blooms were infrequently observed in all other regions during this time, and indeed around the Shetland Islands throughout the whole of 2017. Some PSP toxicity was associated with *Alexandrium* spp. recorded in Loch Melfort (Argyll & Bute), Loch Eishort (Highland: Skye & Lochalsh), Loch Torridon and Loch Glencoul (Highland: Ross & Cromarty) and Loch Laxford and Kyle of Tongue (Highland: Sutherland), all in June and early July. PSP toxins above MPL were associated with *Alexandrium* spp. blooms in Loch Stockinish and Seilebost (Lewis & Harris), also in June and July.
- An extended bloom of *Alexandrium* spp. was observed in Loch Creran (Argyll & Bute), where cell counts were recorded at or exceeding the trigger level for a continuous period of thirteen weeks, from mid June to early September. A maximum bloom density of 5,800 cells/L was detected on 19th July, very similar to the value of 5,860 cells/L reported on 18th July 2016 for this site. Extended bloom periods were also noted at other sites including Loch Glencoul (Highland: Sutherland) for fifteen consecutive weeks from May to August, and Loch Leurbost and Loch Roag: Linngeam (Lewis & Harris), where cell counts exceeded threshold for seventeen and fourteen weeks from late May, respectively.
- Overall, the percentage of samples with *Alexandrium* spp. counts at or above trigger level was lower in May (at 20.5%) compared with the typical average of 33.8% for the years 2007 to 2016. However, the total percentage of *Alexandrium* spp. blooms at or exceeding trigger level during 2017 (19.3%) was similar to that of 2015 (21.7%) and 2016 (21.5%).

Figure 16. Phytoplankton concentrations of *Alexandrium* spp. observed between January and December 2017



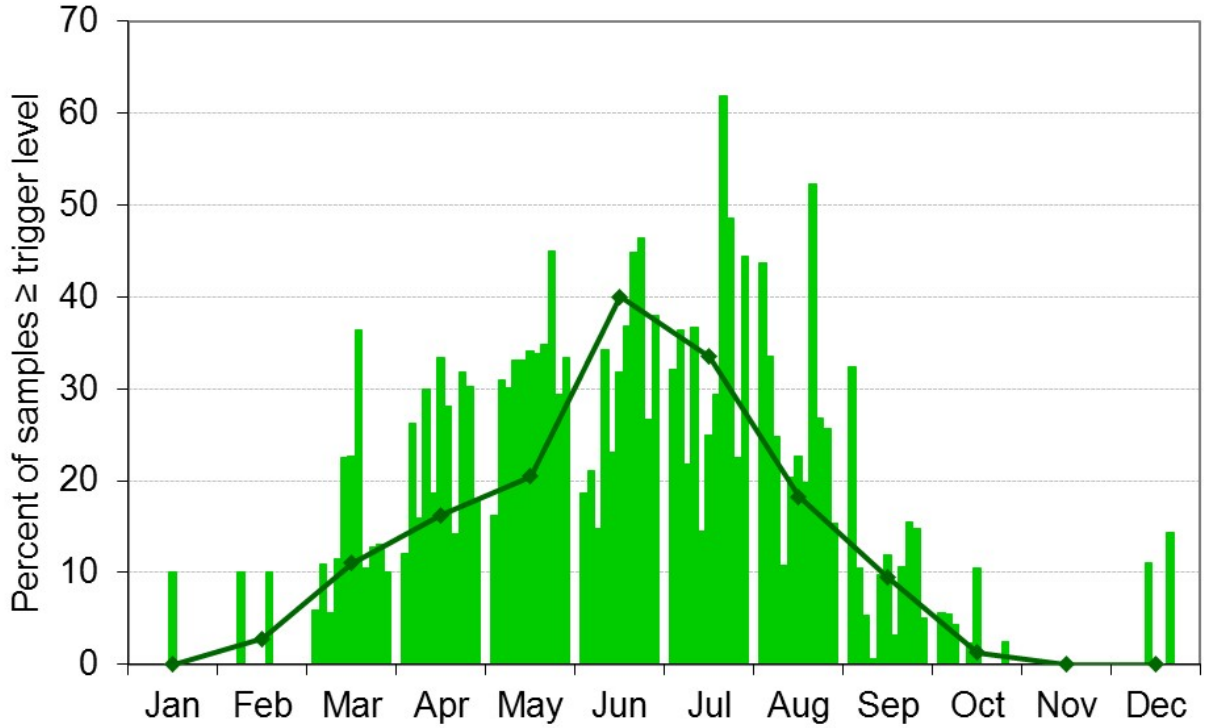


Figure 17: The percentage of samples in which *Alexandrium* spp. equalled or exceeded the trigger level of 40 cells/L in 2017 is indicated by the line. (For comparison, the bars show the percentage of samples in which *Alexandrium* spp. equalled or exceeded the trigger level between 2006 and 2016. NOTE: Data collected prior to July 2014 have been adjusted to the revised trigger level of 40 cells/L for comparative purposes).

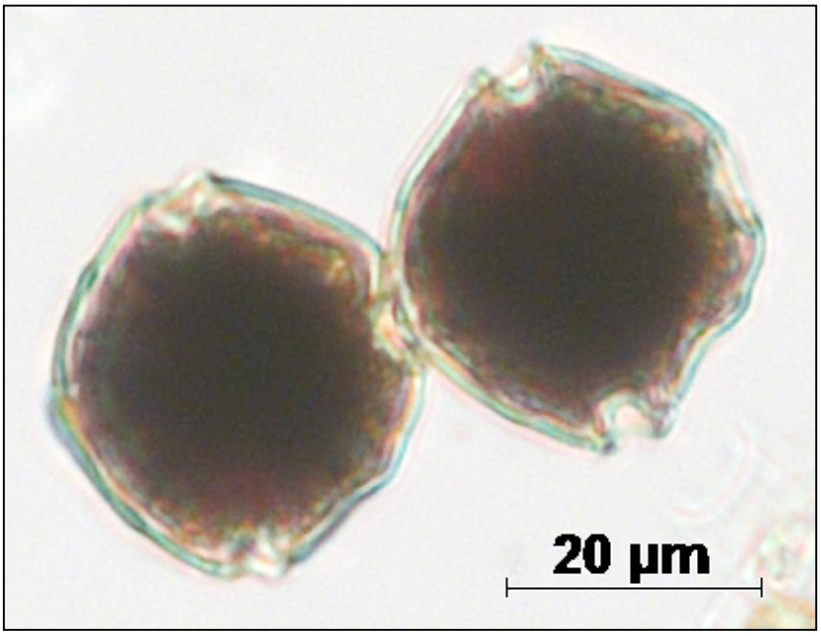


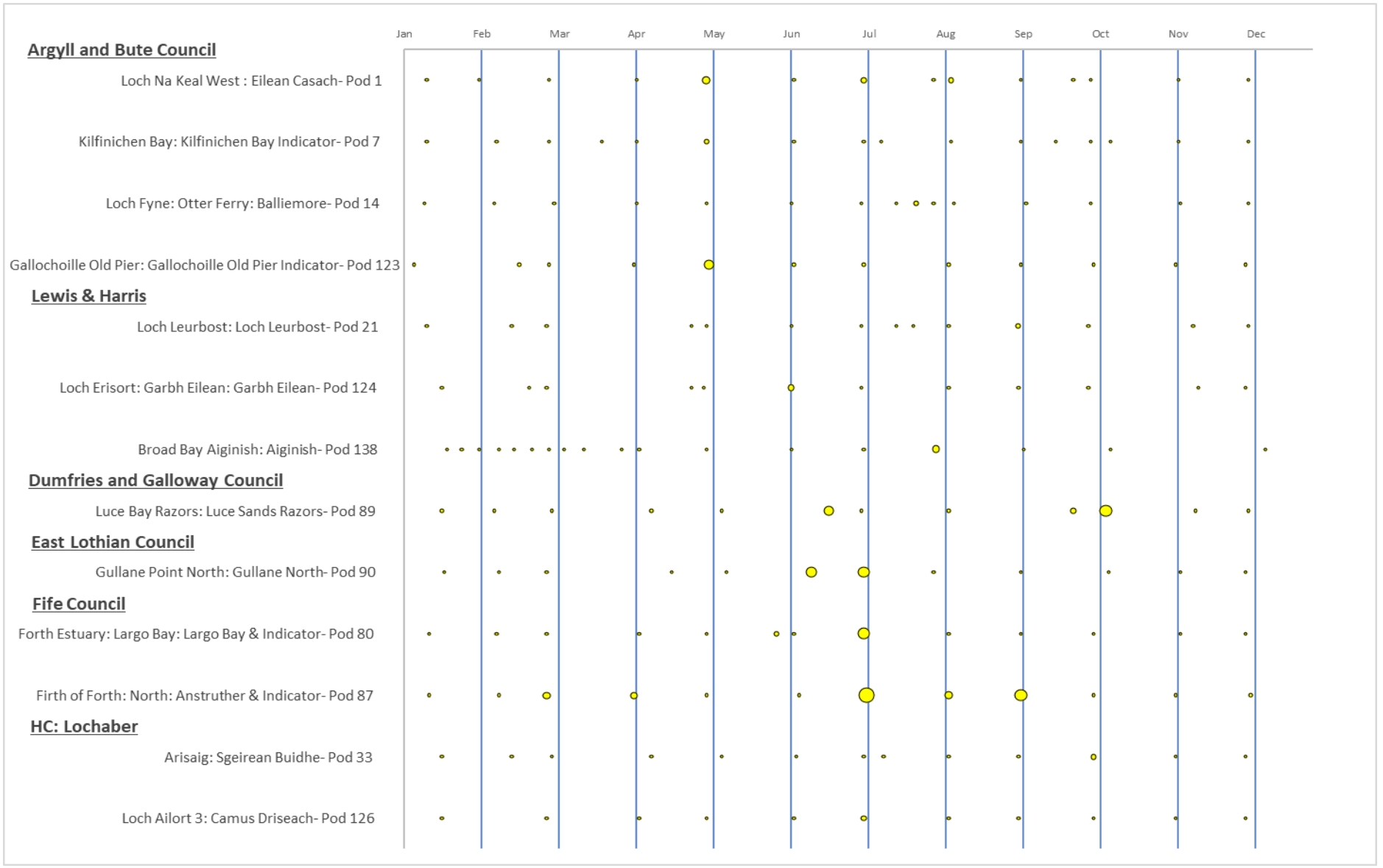
Figure 18: A chain of *Alexandrium* spp. observed at Loch Fyne: Ardkinglas (Argyll & Bute) on 3rd April.

4. Monitoring for ASP toxins

Analyses for amnesic shellfish poisoning (ASP) toxin were conducted on 942 samples from inshore locations and 15 king scallop verification samples collected from commercial establishments. All samples were analysed by an HPLC method. Results are summarised below.

- ASP was detected in 54 inshore samples comprising of: common mussels (25 samples), razors (13), Pacific oysters (11) and surf clams (5).
- These samples originated from 31 sites. Low concentrations were recorded from February through to October 2017, with the peak period occurring between May & September, during which time, ASP was detected in 48 samples (Figure 19).
- No inshore samples exceeded the MPL of 20mg [domoic/epi domoic acid] (DA)/kg shellfish flesh. The highest level recorded was 8.1mg/kg in a mussel sample collected in July 2017**,** originating from Firth of Forth: North – Anstruther (surf clams, Fife Council).
- ASP was detected in five king scallop verification samples from three establishments. Two of these samples comprised of whole king scallop material, the remaining three of shucked product. These shellfish samples were originally harvested in the following offshore scallop grounds; Jura (4 samples) and South Minch (1 sample). Toxin levels ranged between 1.8 and 7.4mg/kg DA/shellfish flesh, with no samples exceeding the MPL.

Figure 19: Concentrations of ASP toxins in sites recording results at quantifiable levels from January to December 2017



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 in sites recording results at quantifiable levels from January to December 2017 (Cont.)

Concentration of ASP toxins:

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

Red = Toxins above MPL
Yellow = Toxins below MPL

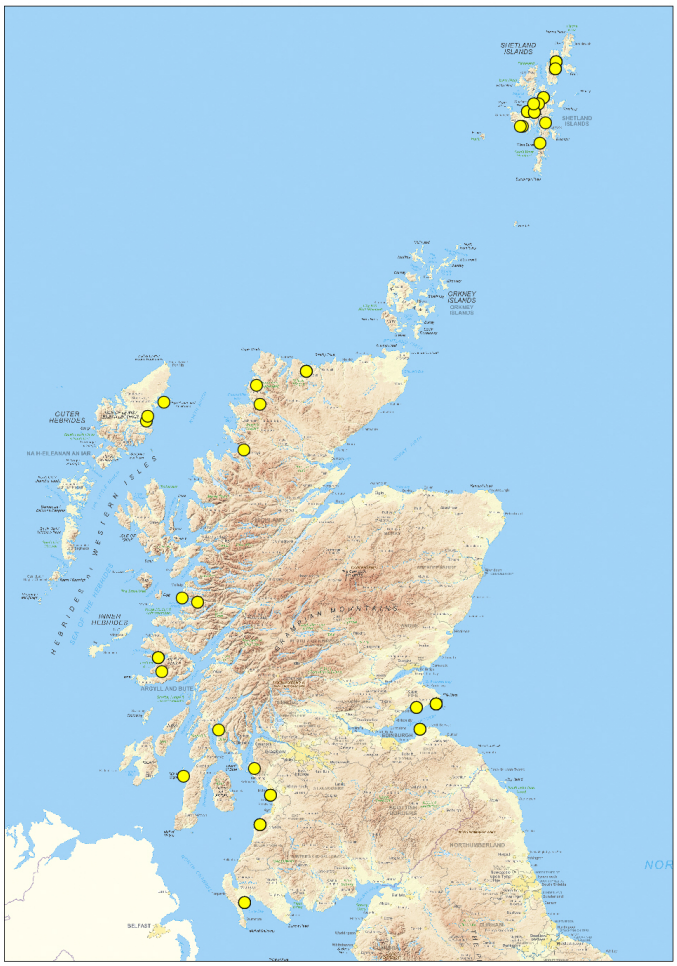


Figure 20: Inshore locations where ASP toxins were detected in 2017 (all below the maximum permitted limit (>20mg/kg))

4.1 Phytoplankton associated with the production of ASP toxins

- Diatoms belonging to the genus *Pseudo-nitzschia* were detected in every month in 2017 (Figure 22) and at all sites and were present in 1,227 (90.8%) of the 1,351 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 July.
- *Pseudo-nitzschia* spp. counts at or above the trigger level (set at 50,000 cells/L) were recorded in 125 samples (9.3%), with 33.5% of the samples analysed in July exceeding this level (Figure 21). The earliest bloom was recorded in Kilfinichen Bay (Argyll & Bute) on 14th March, with an abundance of 102,238 cells/L. The latest bloom of 2017 also occurred in Kilfinichen Bay, with a cell count of 131,509 cells/L reported on 3rd October.
- *Pseudo-nitzschia* spp. blooms were observed around the Shetland Islands (Weisdale Voe, Sandsound Voe and Braewick Voe) in March and early April, but were particularly widespread in July, with low levels of associated ASP toxicity in mussel samples. Cell counts from all the Shetland Islands sites monitored for phytoplankton exceeded trigger level in the second week of July and, following a general decline in cell counts during August, *Pseudo-nitzschia* abundance increased again in September. Diatoms were generally abundant around the Shetland Islands from mid March until late September, possibly related to local upwelling conditions replenishing the supply of nutrients, although the phytoplankton community was dominated by genera other than *Pseudo-nitzschia* until July.
- The densest *Pseudo-nitzschia* spp. blooms were recorded in Aith Voe (Shetland Islands) on 10th July and Loch Roag: Linngeam (Lewis & Harris) on 20th June, where cell counts reached 2,380,351 and 1,979,708 cells/L, respectively. Overall, the percentage of *Pseudo-nitzschia* spp. blooms exceeding trigger level during 2017 (9.3%) was slightly higher than that in 2015 (8.9%) or 2016 (6.2%).

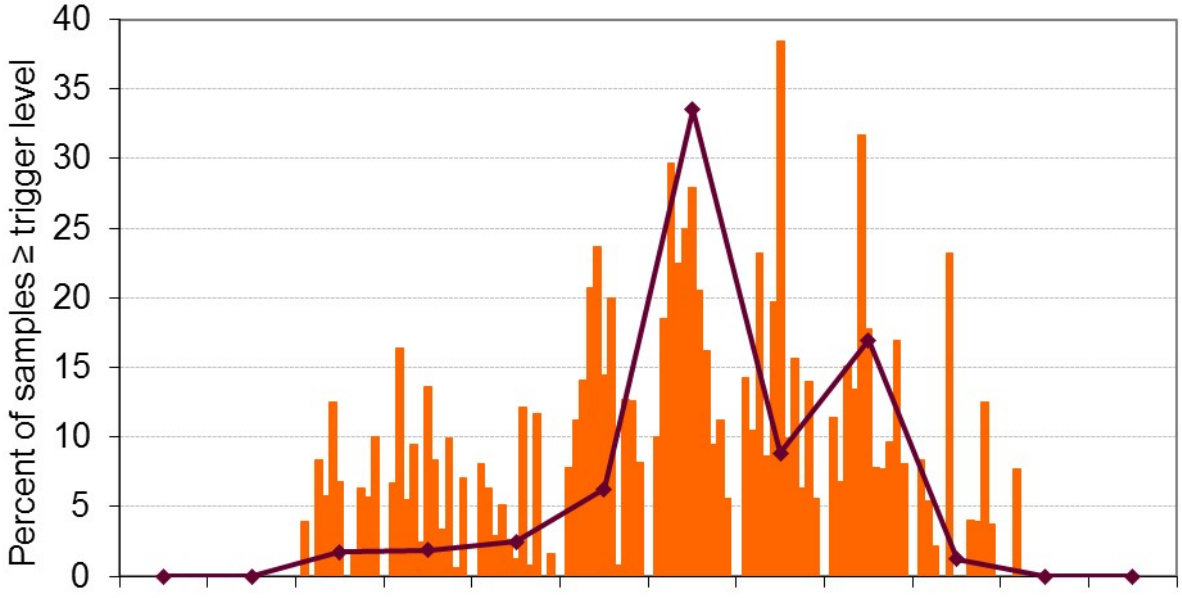
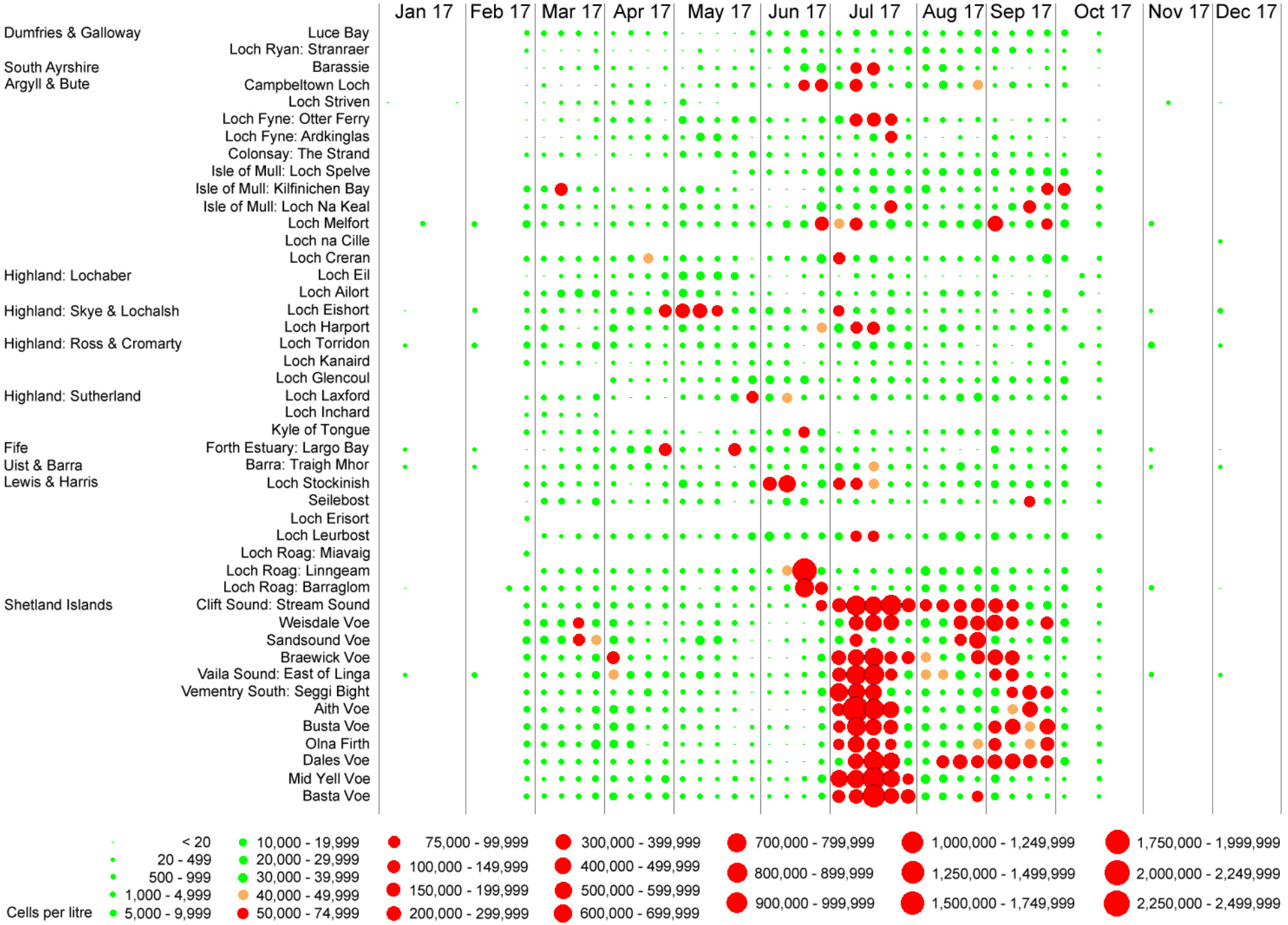


Figure 21: The percentage of samples in which *Pseudo-nitzschia* spp. equalled or exceeded the trigger level of 50,000 cells/L in 2017 is indicated by the line. (For comparison, the bars show the percentage of samples in which *Pseudo-nitzschia* spp. equalled or exceeded the trigger level between 2006 and 2016)**.**

Figure 22: Phytoplankton concentrations of *Pseudo-nitzschia* spp. observed between January and December 2017



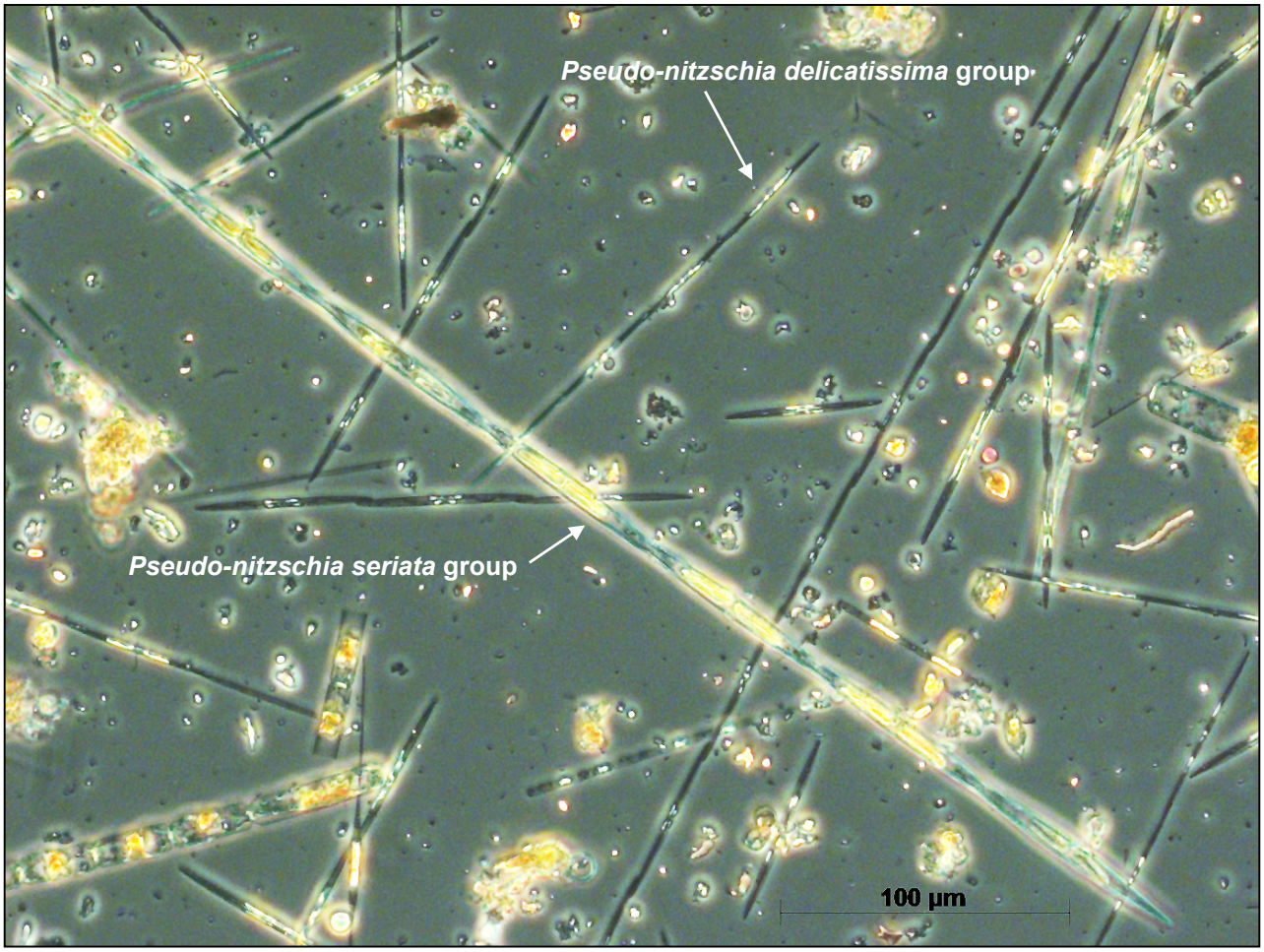


Figure 23: Chains of *Pseudo-nitzschia* spp. observed in Loch Roag: Linngeam (Lewis & Harris) on 20th June. The bloom was composed of approximately 98% *Pseudo-nitzschia delicatissima* group cells and the density exceeded 1.9 million cells/L.

5. Other potentially harmful phytoplankton

The dinoflagellate *Prorocentrum cordatum* was detected in 826 samples analysed in 2017 (61.1%). It was observed from February through to December and was most abundant in May and June, being recorded in 88.5% and 80.0% of the samples analysed, respectively. The densest blooms of 2017 occurred around the Shetland Islands on 26th June, with concentrations of 1,126,073 cells/L recorded in Aith Voe and 463,514 cells/L in Vementry South. Elsewhere in Scotland, less dense blooms were reported in Fife, South Ayrshire, Skye & Lochalsh and Argyll & Bute. No trigger level has been set for *Prorocentrum cordatum*.

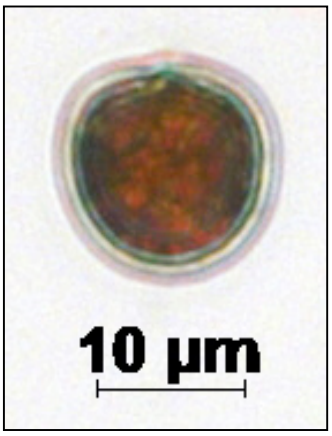


Figure 24: *Prorocentrum cordatum* observed in Loch Inchard (Highland: Sutherland) on 28th February.

The potentially problematic dinoflagellate *Karenia mikimotoi* was not observed in densities likely to negatively impact aquaculture during 2017, but was detected in 183 (13.6%) 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 of the order of several million cells/L may result in both fish and invertebrate mortality due to hypoxia. Cell counts were low in 2017, with a maximum density of 1,940 cells/L recorded in Loch Torridon (Ross & Cromarty) on 18th July.

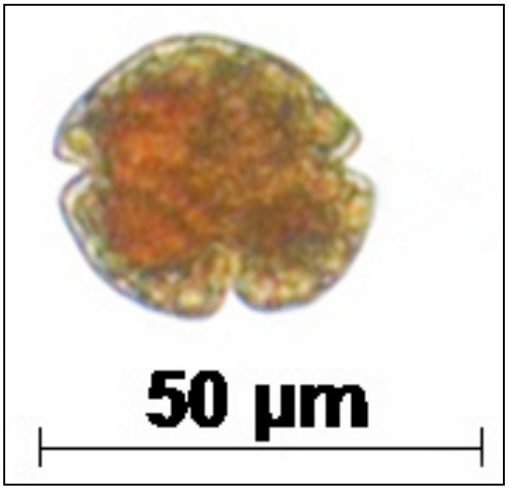


Figure 25: *Karenia mikimotoi* in Loch Na Keal (Argyll & Bute) on 18th April.

6. 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
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

7. Results of the wild pectinidae onshore verification programme

ASP, PSP and LTs analyses were performed on 15 samples from seven 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 26.

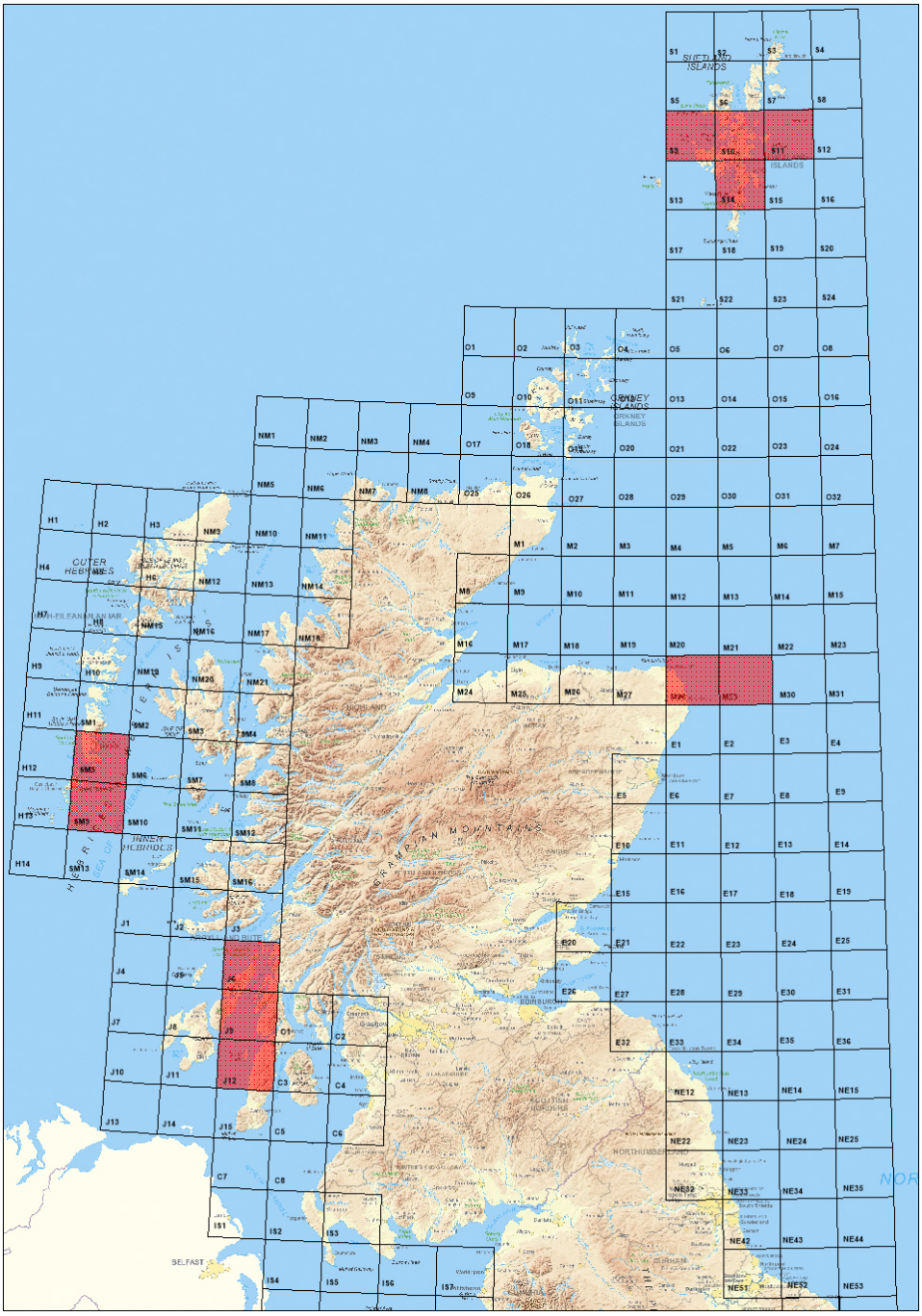


Figure 26: Origins of the wild pectinidae samples received via the FSS onshore official control verification programme in 2017

ASP results

- ASP was detected in five king scallop verification samples from three establishments. Two of these samples comprised of whole king scallop material, the remaining three of shucked product. These shellfish samples were originally harvested in the following offshore scallop grounds; Jura (4 samples) and South Minch (1 sample). Toxin levels ranged between 1.8 and 7.4mg/kg DA/shellfish flesh, none of which exceeded the MPL.

Lipophilic toxin results

- No lipophilic toxins were detected in any scallop verification samples in 2017.

PSP results

- No quantifiable levels of PSP toxins were detected in any scallop verification samples in 2017.

8. Biotoxin Methodology

8.1 Shellfish collection

Inshore Monitoring Programme (classified shellfish production areas):

For the monitoring period of 1st January to 31st December 2017, 2,161 bivalve shellfish samples from 88 inshore sampling locations were submitted for toxin analyses. These sampling locations covered 78 pods within 10 Local Authority regions (16 regional offices).

The inshore samples received by Cefas during the reporting period comprised of mussels (*Mytilus* spp.) (1,525 samples – 70.6% of all samples), Pacific oysters (*Crassostrea gigas*) (399 – 18.5%), razors (*Ensis* spp.) (142 – 6.6%), common cockles (*Cerastoderma edule*) (60 – 2.8%), surf clams (*Spisula solida*) (31 – 1.4%) and native oysters (*Ostrea edulis*) (4 - 0.2%).

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, 11.5% 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 & percentage		No. unverified samples received & percentage	
Angus Council	Hall Mark Meat Hygiene	4	0	0.0%	4	100.0%
Argyll & Bute Council	Argyll & Bute Council	543	543	100.0%	0	0.0%
Comhairle nan Eilean Siar: Lewis & Harris	Hall Mark Meat Hygiene	262	244	93.1%	18	6.9%
Comhairle nan Eilean Siar: Uist & Barra	Hall Mark Meat Hygiene	77	60	77.9%	17	22.1%
Dumfries & Galloway Council	FSS Operations	50	26	52.0%	24	48.0%
East Lothian Council	Hall Mark Meat Hygiene	16	0	0.0%	16	100.0%
Fife Council	Hall Mark Meat Hygiene	91	30	33.0%	61	67.0%
Highland Council: Lochaber	Highland Council	155	85	54.8%	70	45.2%
Highland Council: Ross & Cromarty	Highland Council	60	60	100.0%	0	0.0%
Highland Council: Skye & Lochalsh	Highland Council	83	80	96.4%	3	3.6%
Highland Council: Sutherland	Highland Council	129	129	100.0%	0	0.0%
North Ayrshire Council	FSS Operations	27	25	92.6%	2	7.4%
Orkney Islands Council	Hall Mark Meat Hygiene	0	0		0	
Shetland Islands Council	Hall Mark Meat Hygiene	636	627	98.6%	9	1.4%
South Ayrshire Council	FSS Operations	28	3	10.7%	25	89.3%
Totals		**2,161**	**1,912**	88%	**249**	12%

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	60	0	0.0%
Common mussels	Aquaculture	1525	79	5.2%
Common mussels	Wild harvest	0	0	
Pacific oysters	Aquaculture	399	3	0.8%
Razors	Wild harvest	142	133	93.7%
Surf clams	Wild harvest	31	31	100.0%
Native oysters	Wild harvest	4	3	75.0%

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 (~84 and ~15%, 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 2017

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
Angus Council	4	1	3	0	0
Argyll & Bute Council	543	489	52	2	0
Comhairle nan Eilean Siar: Lewis & Harris	262	226	36	0	0
Comhairle nan Eilean Siar: Uist & Barra	77	46	30	1	0
Dumfries & Galloway Council	50	37	13	0	0
East Lothian Council	16	10	6	0	0
Fife Council	91	61	30	0	0
Highland Council: Lochaber	155	86	62	7	0
Highland Council: Ross & Cromarty	60	54	5	1	0
Highland Council: Skye & Lochalsh	83	70	12	1	0
Highland Council: Sutherland	129	116	13	0	0
North Ayrshire Council	27	26	1	0	0
Orkney Islands Council	0	0	0	0	0
Shetland Islands Council	636	582	41	13	0
South Ayrshire Council	28	15	13	0	0
Totals	**2161**	**1819 (84.2%)**	**317 (14.7%)**	**25 (1.2%)**	**0**

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=25) being received three or four working days post collection throughout this reporting period. None of these late samples were rejected as unsuitable for analyses (see section 4.2).

Wild pectinidae – Onshore Surveillance Programme:

Fifteen king scallop samples (comprising of shucked product (n=13) or whole shellfish (n=2) were collected from seven separate premises by authorised officers from four LA regions (Argyll & Bute, Comhairle nan Eilean Siar: Uist & Barra, Shetland Isles and South Ayrshire Council) during the reporting period and submitted to Cefas for toxin analyses.

The scallop samples were originally harvested from the following offshore scallop grounds: Jura (J06, J09 & J12), Shetlands (S09, S10, S11 & S14), Moray (M28 & M29) and South Minch (SM05 & SM09) (Figure 26, page 35).

Twelve samples arrived within one working day of collection, with three samples received two working days post collection.

8.2 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 six inshore samples were rejected in 2017. 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 2017.

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)
Angus Council	4	0	0
Argyll & Bute Council	543	0	1
Comhairle nan Eilean Siar: Lewis & Harris	262	0	0
Comhairle nan Eilean Siar: Uist & Barra	77	0	0
Dumfries & Galloway Council	50	1	0
East Lothian Council	16	0	0
Fife Council	91	0	0
Highland Council: Lochaber	155	0	0
Highland Council: Ross & Cromarty	60	0	0
Highland Council: Skye & Lochalsh	83	0	1
Highland Council: Sutherland	129	1	1
North Ayrshire Council	27	0	0
Orkney Islands Council	0	0	0
Shetland Islands Council	636	0	0
South Ayrshire Council	28	1	0
Totals	**2161**	**3 (0.14%)**	**3 (0.14%)**

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.

Methodology of shellfish analysis

The methods used for routine toxin analysis of shellfish were those specified by FSS 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 and conforms to the performance characterisitcs 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 2017

Toxin group	Methods employed	Species tested	Dates	Accreditation status (as of 31st December 2017,) to ISO 17025:2005 standard
ASP	LC-UV	All species	1st January to 31st December 2017	Accredited
PSP	LC-FLD (screen, semi-quantitative screen & full quantitation)	All species	1st January to 31st December 2017	Accredited
Lipophilic toxins	LC-MS/MS	All species	1st January to 31st December 2017	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)

8.2 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 97% of all tests carried out within one working day of receipt and 100% within two working days (Table 8).

Of the 90 samples results which were reported after one working day of receipt, 53 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	FSS specified targets	Laboratory statistics in the reporting period (all results combined)
ASP by HPLC	90% within 1 working day 98% within 3 working days	97% within 1 working day 100% within 2 working days
Lipophilic toxins by LC-MS	90% within 1 working day 98% within 3 working days	
PSP by HPLC (screen)	90% within 1 working day 98% within 3 working days	
PSP by HPLC (quantitation)	90% within 2 working days 98% 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 2017 is given in Table 9.

Table 9: Turnaround times, by Local Authority region, for samples received from inshore areas in 2017

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
Angus Council	4	4	0	0
Argyll & Bute Council	543	524	19	0
Comhairle nan Eilean Siar: Lewis & Harris	262	241	21	0
Comhairle nan Eilean Siar: Uist & Barra	77	74	3	0
Dumfries & Galloway Council	50	49	1	0
East Lothian Council	16	16	0	0
Fife Council	91	91	0	0
Highland Council: Lochaber	155	148	7	0
Highland Council: Ross & Cromarty	60	55	5	0
Highland Council: Skye & Lochalsh	83	78	5	0
Highland Council: Sutherland	129	115	14	0
North Ayrshire Council	27	27	0	0
Orkney Islands Council	0	0	0	0
Shetland Islands Council	636	622	14	0
South Ayrshire Council	28	28	0	0
Totals	**2161**	**2072 (95.9%)**	**89 (4.1%)**	**0**

9. Phytoplankton Methodology

9.1 Water collection

For the monitoring period 1st January to 31st December 2017, a total of 1,352 seawater samples were collected from 45 sampling locations within seven Local Authority regions (eleven 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	305	
Comhairle nan Eilean Siar: Lewis & Harris	Hall Mark Meat Hygiene	169	
Comhairle nan Eilean Siar: Uist & Barra	Hall Mark Meat Hygiene	37	
Dumfries & Galloway Council	FSS Operations	66	
Fife Council	Hall Mark Meat Hygiene	38	1
Highland Council: Lochaber	Highland Council	66	
Highland Council: Ross & Cromarty	Highland Council	69	
Highland Council: Skye & Lochalsh	Highland Council	70	
Highland Council: Sutherland	Highland Council	99	
Shetland Islands Council	Hall Mark Meat Hygiene	400	
South Ayrshire Council	FSS Operations	33	
TOTALS		1352	1

Samples were collected and packaged in accordance with SRSL's guidance and protocols and sent to the SRSL Oban laboratory for analysis. One sample was not analysed as it was not required, due to the reduced autumn sampling schedule. This resulted in a total of 1,351 samples being analysed between 1st January and 31st December 2017.

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 (98.8%) arrived at the laboratory within one or two working days of sample collection, 89.0% and 9.8%, respectively (Table 11). Of the samples taking

more than one working day to arrive, 87.8% were from remote areas. In addition, a road closure of the A85 into Oban due to a chemical spill was responsible for the delayed receipt of 18 samples.

Table 11: Number of phytoplankton samples received from each Local Authority region and time taken between collection and receipt at SRSL in 2017.

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	305	272	30	2	1
Comhairle nan Eilean Siar: Lewis & Harris	169	151	18	0	0
Comhairle nan Eilean Siar: Uist & Barra	37	30	6	1	0
Dumfries & Galloway Council	66	60	6	0	0
Fife Council	38	33	5	0	0
Highland Council: Lochaber	66	55	11	0	0
Highland Council: Ross & Cromarty	69	62	7	0	0
Highland Council: Skye & Lochalsh	70	55	14	1	0
Highland Council: Sutherland	99	93	5	1	0
Shetland Islands Council	400	361	29	10	0
South Ayrshire Council	33	32	1	0	0
TOTAL SAMPLES	**1,352**	**1,204**	**132**	**15**	**1**
Percentage of total		**89.0%**	**9.8%**	**1.1%**	**0.07%**

9.2 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 27), but if the amount of sediment present in the sub-sample is excessive, 25 mL or 10 mL sub-samples may be used.

Figure 27: 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. However, sufficient data are not always available to allow trigger levels to be set for all the target harmful algal species. Trigger levels remained at the same cell concentrations as used in 2015 (Table 7, Page 42).

9.3 Reporting of results

Upon completion of analyses, results were collated and quality control checked prior to submission to the FSS. During 2017, 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.

10. References:

AOAC International. (2005). AOAC Official method 2005.06 Quantitative determination of Paralytic Shellfish Poisoning Toxins in shellfish using pre-chromatographic oxidation and liquid chromatography with fluorescence detection. Gaithersburg, MD, USA: AOAC International.

European Communities (2004). Regulation (EC) 853/2004 of the European Parliament and of the Council of 29th April 2004 laying down the specific hygiene rules for the hygiene of foodstuffs.

European Communities (2004). Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29th April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

European Communities (2005). Regulation (EC) 2074/2005 of the European Parliament and of the Council of 5th December 2005 which lays down the implementing measures for certain products under Regulation (EC) 853/2004 and for the organisation of official controls under Regulation (EC) 854/2004 and 882/2004, derogating from Regulation (EC) No 852/2004 and amending Regulations (EC) Nos 853/2004 and 854/2004.

European Communities (2004). Regulation (EC) 882/2004 of the European Parliament and of the Council of 29th April 2004, which prescribes requirements for Official Controls performed to ensure the verification of compliance with feed and food law.

Statutory Instruments, (2006). The Food Safety (Fishery products and live shellfish) (Hygiene) Regulations: Schedule 2 (Production and placing on the market conditions for live shellfish) p36-45.

Turner, A.D., Stubbs, B., Coates, L., Dhanji-Rapkova, M., Hatfield, R.G., Lewis, A.M., Rowland-Pilgrim, S., O'Neil, A., Stubbs, P., Ross, S., Baker, C. and Algoet, M. (2014) Variability of paralytic shellfish toxin occurrence and profiles in bivalve molluscs from Great Britain from official control monitoring as determined by pre-column oxidation liquid chromatography and implications for applying immunochemical tests. *Harmful Algae.* **31,** 87-99