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

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Authors: Keith Harrison [1], Alison Walton [2], Lewis Coates [1], Andrew Turner [1], Steve Milligan [2] and Myriam Algoet [1]

1) Cefas Laboratory, Barrack Road, Weymouth, Dorset, DT4 8UB
2) Cefas Laboratory, Pakefield Road, Lowestoft, Suffolk, NR33 0BR

Document prepared by:	Lewis Coates, Keith Harrison & Alison Walton	
Document quality checked by:	Myriam Algoet, 19/02/18 Alison Walton, 04/01/2018	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 the FSA and showing the results of the phytoplankton and toxin analyses undertaken on samples submitted by local authorities. All results were quality checked and approved prior to release to the FSA 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 local authority 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 FSA sampling points.

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1. Summary

This report describes the results of the Official Control Biotoxin Monitoring Programme for England and Wales for the period 1st January to 31st December 2017.

The laboratory testing for biotoxins in shellfish and potentially harmful phytoplankton in water samples, the co-ordination of the programme and its logistics were conducted by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) on behalf of the Food Standards Agency (FSA), the central competent authority for food safety. The programme aimed at delivering the testing required for the statutory monitoring of marine biotoxins in shellfish from classified production and relaying areas in England and Wales, and for identification and enumeration of potentially harmful micro-algae in selected shellfish harvesting areas, as required by EC Regulations 854/2004, 882/2004 and 2074/2005.

In the reported period, 56 of the 57 classified English and Welsh harvesting and relaying areas were monitored (directly or indirectly[1]) from 76 inshore sampling locations (Figures 1 and 2), giving a coverage rate of 98.2%[2]. A total of 862 inshore shellfish samples and 921 phytoplankton samples were submitted for analyses by staff from 39 Local Authorities (LAs).

Table 1: Maximum permitted limits of toxins in shellfish flesh[3]

Toxin	Maximum Permitted Limits
ASP	Exceeding 20 mg [Domoic/epi-domoic acid]/kg [shellfish flesh]
LTs	Diarrhetic shellfish poisoning (DSP) toxins and pectenotoxins (PTX) together, exceeding 160µg [okadaic acid (OA) equivalents]/kg [shellfish flesh] or Yessotoxins, exceeding 3.75mg [yessotoxin (YTX) equivalents]/kg [shellfish flesh] or Azaspiracids, exceeding 160µg [azaspiracid (AZA) equivalents]/kg [shellfish flesh].
PSP	Exceeding 800µg [saxitoxin equivalents (STX di-HCl eq.)]/kg [shellfish flesh]

[1] In this case, the classified production areas were monitored by sampling adjacent areas where appropriate
[2] 2% of the classified production areas were supplying shellfish only for ongrowing
[3] Regulation (EC) 853/2004

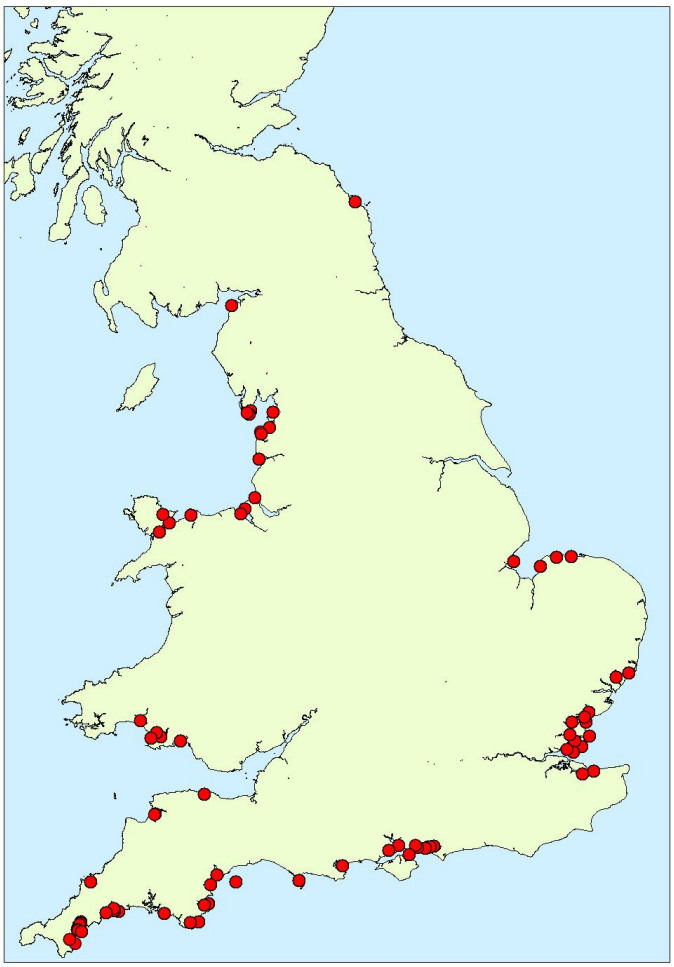


Figure 1. English and Welsh flesh sampling locations – 2017 Biotoxin monitoring programme

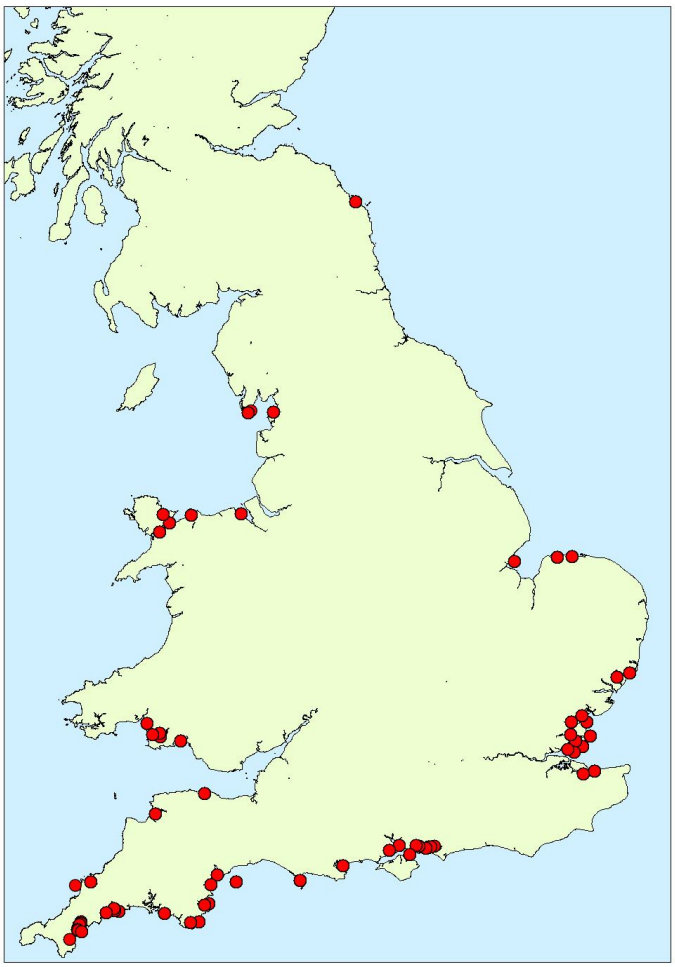


Figure 2. English and Welsh water sampling locations – 2017 Biotoxin monitoring programme

Results of the shellfish monitoring programme for the twelve-month period were as follows (all toxin results stated for Paralytic Shellfish Poisoning (PSP) toxins and Lipophilic Toxins (LTs) refer to the high value calculated from method uncertainty):

- **Amnesic Shellfish Poisoning (ASP) toxins - summary**

746 inshore shellfish samples were tested for ASP toxins using a high-performance liquid chromatography (HPLC) method. ASP toxins were detected in 26 samples from 11 production areas (Figure 3). The greatest proportion of samples containing ASP originated from the south-west of England (17 samples). The shellfish species affected included mussels (2 samples), Pacific oysters (6 samples), cockles (4 samples), hard clams (2 samples) and surf clams (12 samples). None of the inshore shellfish samples tested for ASP exceeded the maximum permitted level (MPL) of 20 mg/kg in 2017. The highest ASP concentration was recorded in March/April (3.8 mg/kg) from the Start Bay production area. Of the 12 samples collected from this area in 2017, all samples contained ASP between 3 and 3.8 mg/kg.

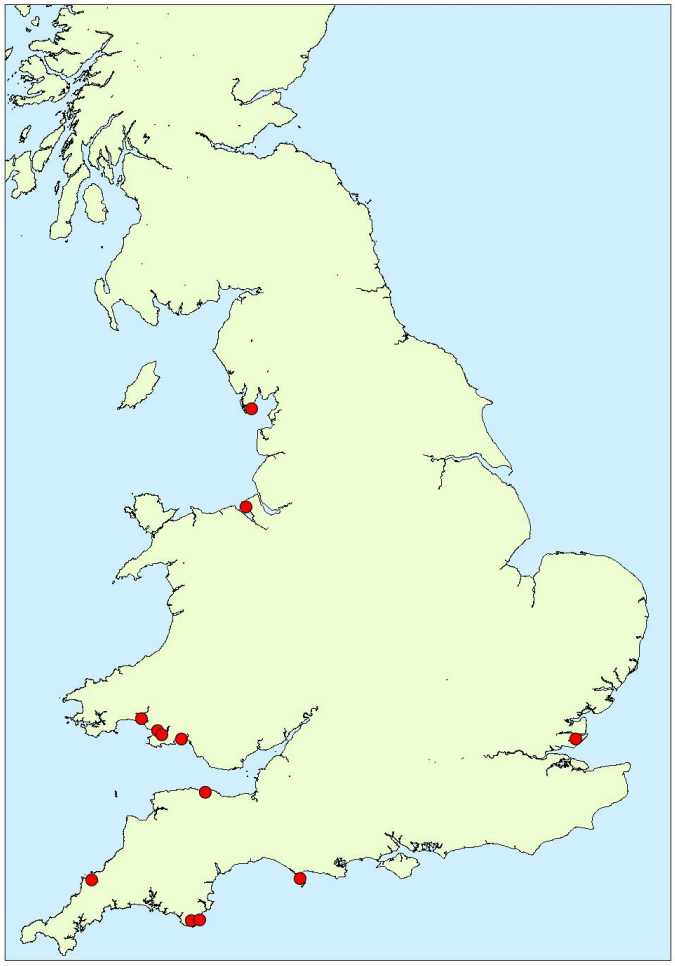


Figure 3: Location of classified production and/or relaying areas where ASP toxins were detected in 2017 (all below the MPL (20 mg [domoic+*epi*-domoic acid]/kg [shellfish tissue])

- **Paralytic Shellfish Poisoning (PSP) toxins - summary**

833 inshore shellfish samples were screened for PSP toxins using the HPLC semi-quantitative method. Four samples also required analysis by the full quantitative method. This is a decrease on the number and levels detected in 2016. Three samples in total recorded PSP toxin levels above the MPL (800µg STX di-HCl eq.)]/kg). These were from the Fowey and Salcombe production areas. Four samples in total recorded PSP toxin levels above the trigger level (400µg STX di-HCl eq.)]/kg). These were from the Pont Pill and Geese Quarries sampling points (Figure 4 and 6).

The Salcombe production area recorded two results above the MPL between 09/08/2017 and 22/08/2017. *Alexandrium* spp. was the predominant toxin producing algal genera in this area, it was first detected on 11/04/2017. *Alexandrium* continued to be detected in 6 further samples in this area through to 20/09/2017. PSP toxins were first detected above the MPL (831 µg/kg) on 09/08/2017. The highest level of PSP toxins (1713 µg/kg) was recorded on 22/08/2017. Toxin levels fell rapidly following this peak. A second consecutive sample recorded below MPL on 20/09/2017 and the site was allowed to reopen. Toxins continued to be detected below MPL until 04/10/2017.

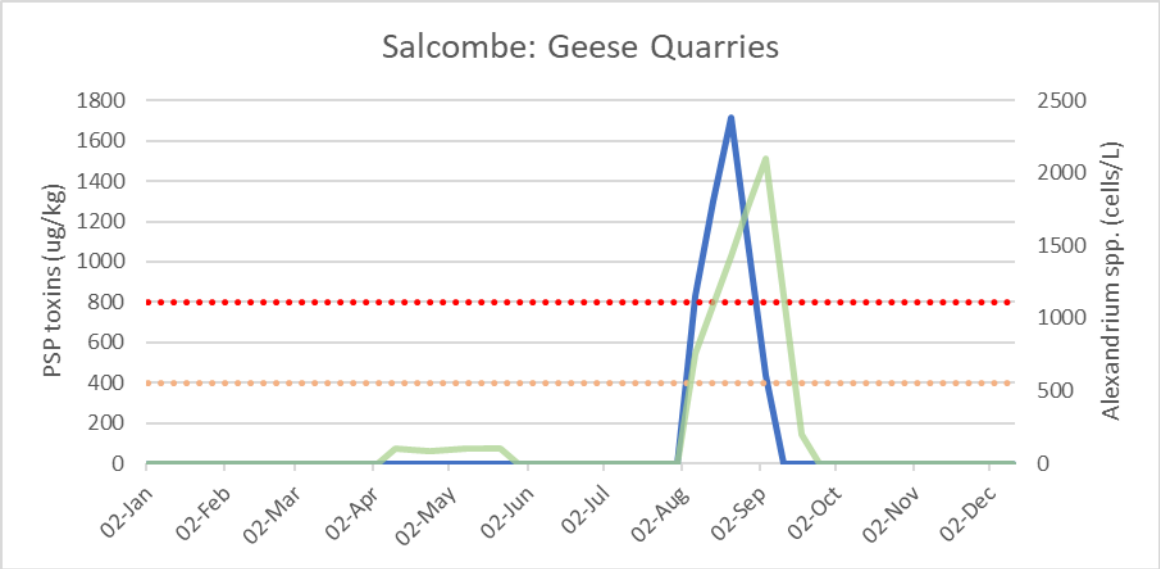


Figure 4. Results of PSP testing (green line) and *Alexandrium* species enumeration (blue line) in Salcombe production area in 2017. (Orange dotted line indicates the resampling trigger level and red dotted line indicates the MPL in flesh).

The Fowey production area recorded one result above the MPL (1590 µg/kg) on 01/08/2017. Prior to this event, *Alexandrium* was first detected on 06/06/2017 and, again from 19/07/2017 through to 01/08/2017, the date when the breach of the toxin MPL occurred. Toxin levels rose sharply and quickly declined during late Summer. A second consecutive sample recorded below MPL on 15/08/2017 and the site was allowed to reopen. Toxins continued to be detected below MPL through to 12/09/2017.

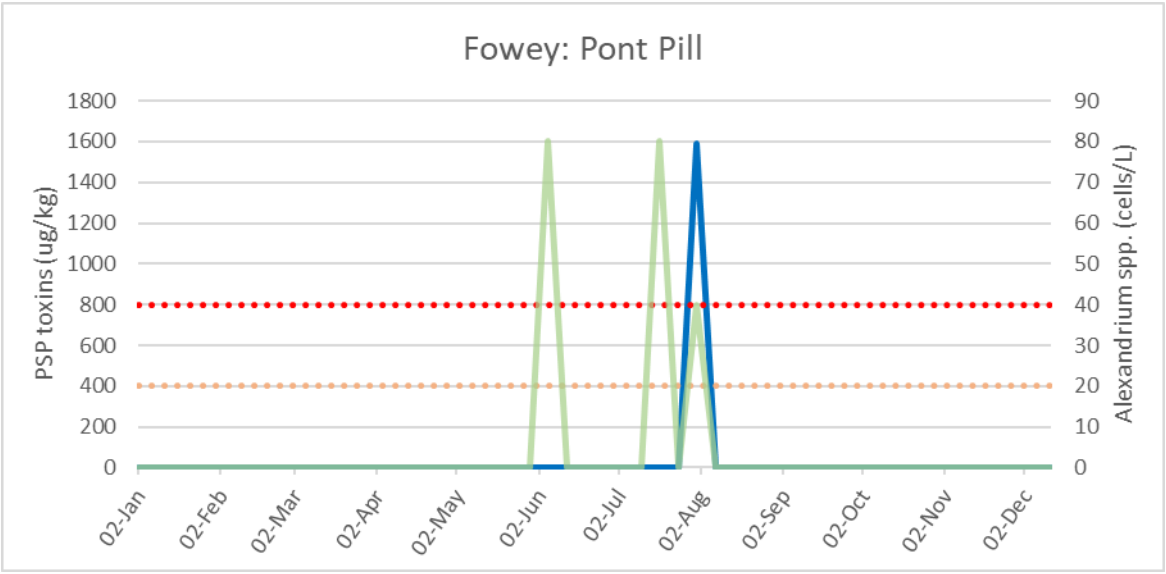


Figure 5. Results of PSP testing (blue line) and *Alexandrium* species enumeration (green line) in Fowey production area in 2017

Figure 6: Location of classified production and/or relaying areas where PSP toxins were detected <u>below</u> the MPL of 800 µg [STX equivalent]/kg [shellfish tissue] in 2017

Figure 7: Location of classified production and/or relaying areas where PSP toxins were detected <u>above</u> the MPL of 800 µg [STX equivalent]/kg [shellfish tissue] in 2017

- **Lipophilic toxins (LTs) - summary**

A total of 762 inshore samples were analysed for LTs using the Liquid Chromatography - tandem mass spectrometry (LC-MS/MS) method. The lipophilic toxins are sub-divided into three regulated groups.

Yessotoxins (YTXs)
Not detected in any samples received in 2017.

Azaspiracid group toxins (AZAs)
Not detected in any samples received in 2017.

Okadaic Acid/Dinophysistoxins/Pectenotoxins (OA/DTX/PTX)

Detected in 40 samples from 10 production areas (Figure 8). This is the lowest number of recorded instances of this toxin group in inshore shellfish samples in the last four years. Four mussel samples from one production area (Lyme Bay) contained OA/DTX/PTXs above the MPL (set at 160 µg OA eq/kg) (Table 1 & Figure 9).

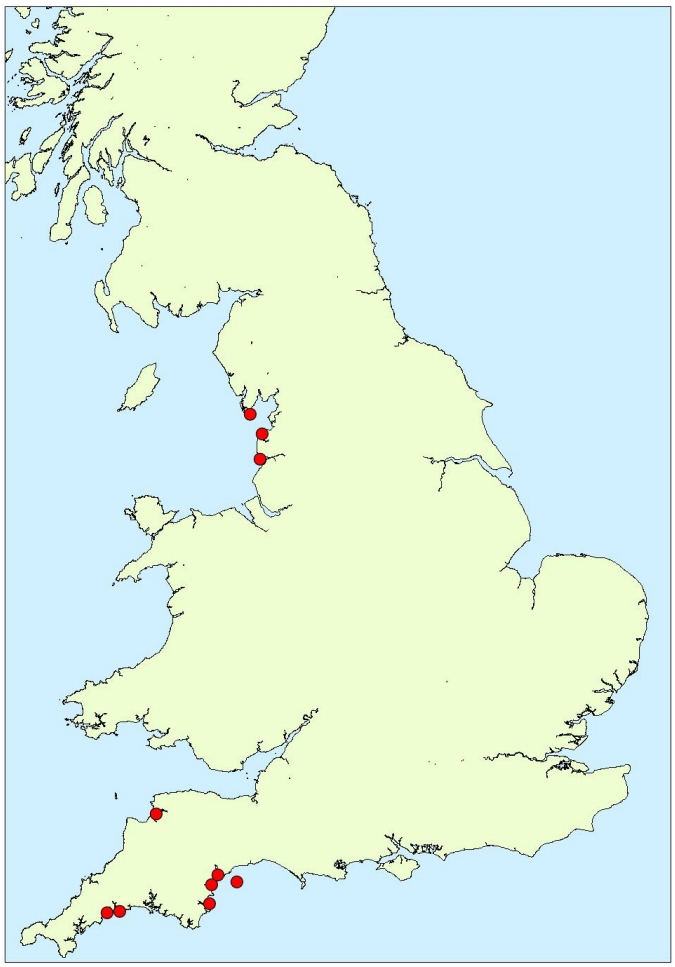


Figure 8: Location of classified production and/or relaying areas where OA/DTXs/PTXs group toxins were detected <u>below</u> the MPL of 160 µg [OA equivalent]/kg [shellfish tissue] in 2017

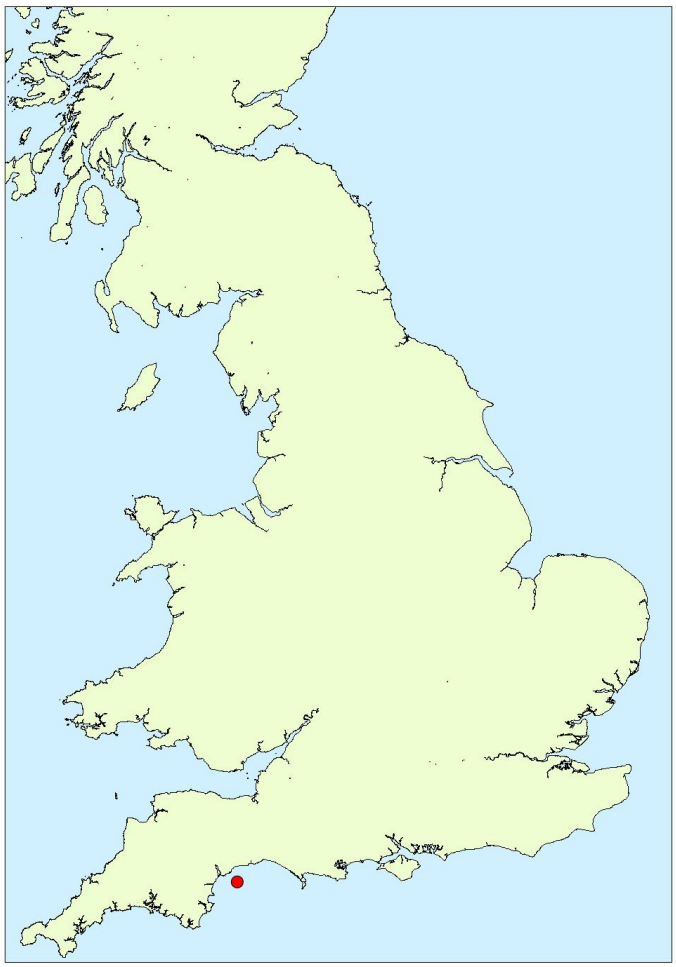


Figure 9: Location of classified production and/or relaying areas where OA/DTXs/PTXs group toxins were detected <u>above</u> the MPL of 160 µg [OA equivalent]/kg [shellfish tissue] in 2017

The Lyme Bay production area recorded four consecutive results above the MPL in samples collected between 12/07/2017 and 01/08/2017. The highest concentration during this event was recorded in a sample collected on 12/07/2017 (236 µg/kg). The second consecutive result below the MPL was recorded in a sample collected on 16/08/2017, however detection of toxins continued until mid-September. This toxin group appeared at a similar time in 2016, although peak concentrations and the number of results above the MPL were lower in 2017.

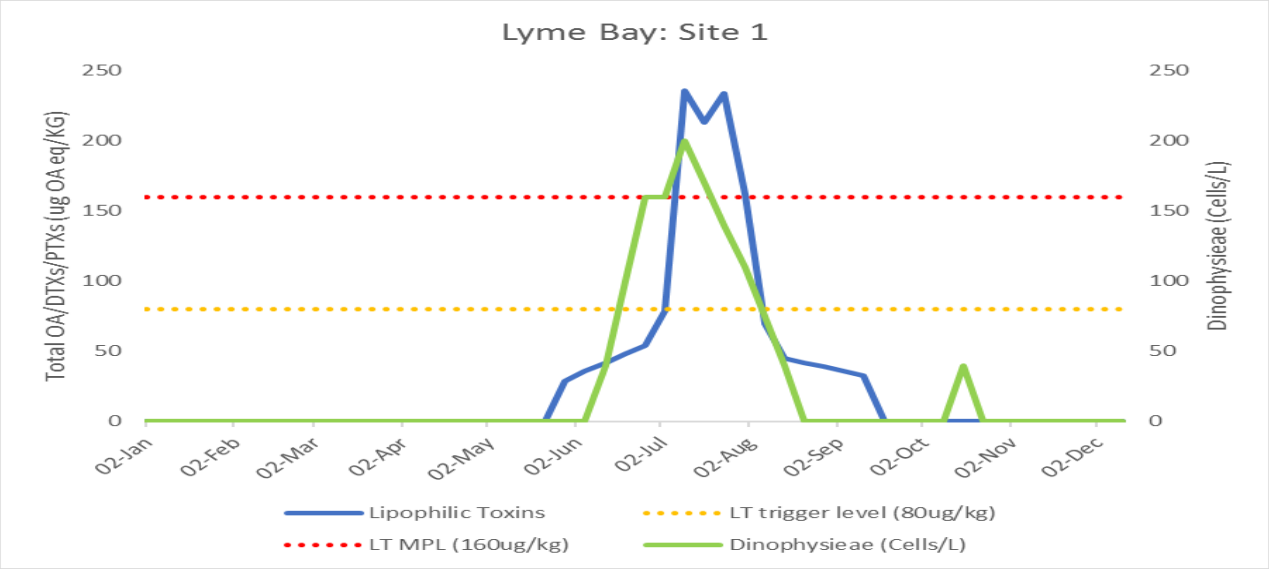


Figure 10: Results of LT testing and Dinophysieae enumeration in Lyme Bay production area in 2017.

Toxin	Samples where toxin levels exceeded the maximum permitted level (ASP: > 20 mg [domoic +*epi*-domoic acid]/kg [shellfish flesh]; OA/DTXs/PTXs: >160 µg [OA eq.]/kg [shellfish flesh]; AZAs: >160 µg [AZA1 eq.]/kg [shellfish flesh]; YTXs: >3.75 mg [YTX eq.]/kg [shellfish flesh]; PSP: > 800 µg [STX eq.]/kg [shellfish flesh])			
	Local Authority	**Production area & site**	**Date samples collected**	**Highest value reported (Shellfish species)**
ASP	None	None	None	None
OA/DTXs/ PTXs	Torbay BC	Lyme Bay: Site 1	12/07/2017 to 01/08/2017: 4 samples over this period	236 µg/kg (Mussels)
AZAs	None	None	None	None
YTXs	None	None	None	None
PSP	Cornwall PHA	Fowey: Pont Pill	01/08/2017	1590 µg/kg (Mussels)
	South Hams DC	Salcombe: Geese Quarries	09/08/2017 to 22/08/2017: 2 samples over this period	1713 µg/kg (Pacific Oysters)

Table 2: Summary of sites where either ASP, PSP or lipophilic toxins were detected above the maximum permitted limits in 2017.

- **Insufficient/unsuitable samples**

Three shellfish samples (0.3% of all samples submitted) were rejected on the grounds of being submitted outside the scheduled testing frequency.

Phytoplankton monitoring - summary

The results of the phytoplankton monitoring of classified production and relaying areas in England and Wales for 2017 are summarised below. Where the stated trigger levels (see Appendix 2, Table 1) were exceeded, additional flesh and water samples were requested the following week.

- *Pseudo-nitzschia* species (ASP toxin producer) were recorded in 590 samples from 50 production areas. The trigger level (set at 150,000 cells/L) was exceeded on 12 occasions from 6 production areas (Table 3, Figure 11). The highest cell density was recorded in a sample from Lantivet Bay: Sandheap Point collected on 15 August (893,000 cells/L). The number of samples which exceed the trigger level for *Pseudo-nitzschia* species has fluctuated considerably from year to year. There has been a decrease in the number of breaches compared to 2016, but is similar to the number seen in 2015.

- *Alexandrium* species (PSP toxins producers) were recorded in 50 samples from 22 production areas (Table 3, Figure 12), representing a decrease in the occurrence of this genus compared to last year. Recorded maximum cell density was also less than last year, with a density of 2100 cells/L recorded from Salcombe: Geese Quarries. These levels are comparable to those recorded over the period 2013 to 2015, when annual recorded occurrences did not exceed 55 samples, and maximum cell densities did not exceed 27,000 cells/L in each year. In contrast, last year, annual recorded occurrences were from 107 samples, and maximum recorded cell densities was 13,617,000 cells/L. This was also from Salcombe: Geese Quarries.

- Dinophysiaceae (lipophilic toxins producers) were recorded in 75 samples from 28 production areas. The trigger level (set at 100 cells/L) was exceeded by 21 samples from 12 production areas (Table 3, Figure 13). This is a notable decrease (-80.2%) in the number of Dinophysiaceae trigger level breaches compared to 2016. The maximum cell density recorded in 2017 was 320 cells/L from Lantivet Bay: Sandheap Point in Cornwall, the same site as the highest Pseudo-nitzschia cell density, though at a different sampling time. This decrease is mostly due to fact that additional water sampling procedures had changed, with no water samples being collected when the beds were closed due to high levels of toxins in the flesh, resulting in weekly flesh testing.

- *Prorocentrum lima* (lipophilic toxins producers) were detected in 9 samples from 7 production areas (Table 3, Figure 14). The trigger level (set at 100 cells/L) was exceeded by just 2 samples. The highest cell density was 200 cells/L in a sample from Conwy: Conwy West. *Prorocentrum lima* is considered an epi-benthic species, and it is likely that its detection in the water column is associated with sediment disturbance.

Taxa with trigger levels (cells/L)	2017 Occurrences	2017 Breaches	% change in breach numbers compared to 2016	2017 max. recorded density (cells/L)
Alexandrium (presence)	50	50	-53.3%	2,100
Pseudo-nitzschia (150,000)	590	12	-55.6%	893,000
Dinophysiaceae (100)	75	21	-80.2%	320
Prorocentrum lima (100)	9	2	0.0%	200

Table 3: Summary of Phytoplankton taxa with trigger levels

- *Prorocentrum cordatum* were recorded in 168 samples from 40 production areas. These figures show an increase in occurrence but with cell densities peaking in June at 4000 cells/L, which was similar to that in 2016.
- *Lingulodinium polyedrum* were recorded in 2 samples from 2 production areas. The maximum recorded cell density was 200 cells/L from Lune - Wyre BC: Sea Centre South.
- *Protoceratium reticulatum* were recorded in a single sample from Burry Inlet - Carmarthenshire CC: Machynys at just 40 cells/L. Both *P. reticulatum* and *L. polyedrum* have typically been recorded at relatively low frequencies and densities in samples from English and Welsh shellfish production areas over the last twelve years.

Of the 921 phytoplankton samples submitted in 2017, 9 (0.94%) were rejected for analysis, 6 due to high sediment. This is a reduction of 1% in the number of high sediment samples compared to 2016, and is the lowest number of samples rejected due to high sediment since 2006. A further 3 samples were not analysed for incorrect frequency of submission.

Figure 11: Locations of sites where *Pseudo – nitzschia* species were detected above trigger level in 2017

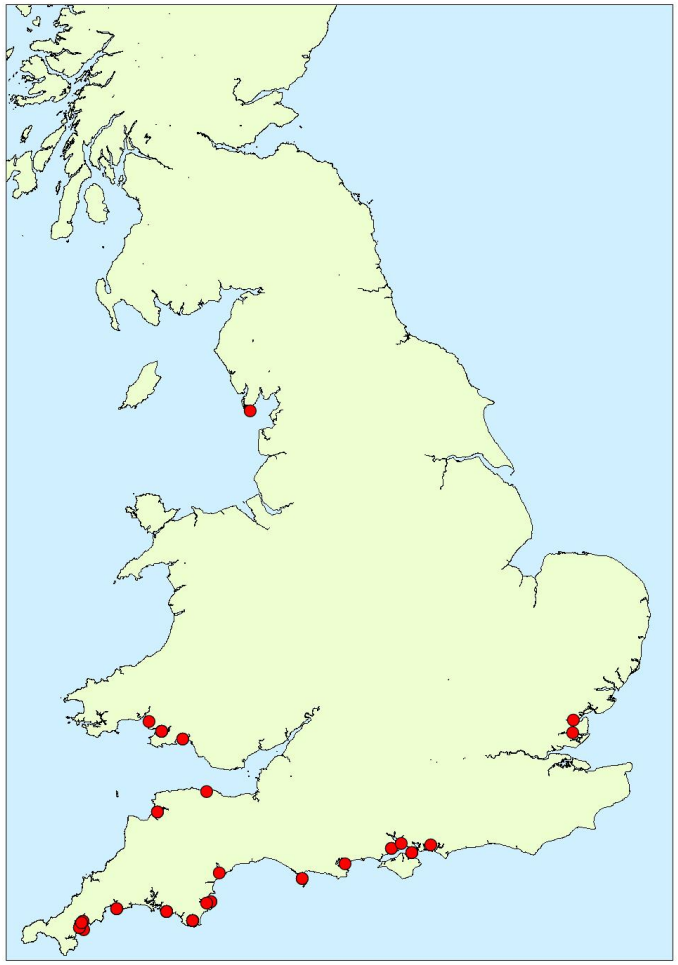


Figure 12: Locations of sites where *Alexandrium* species were detected above trigger level in 2017

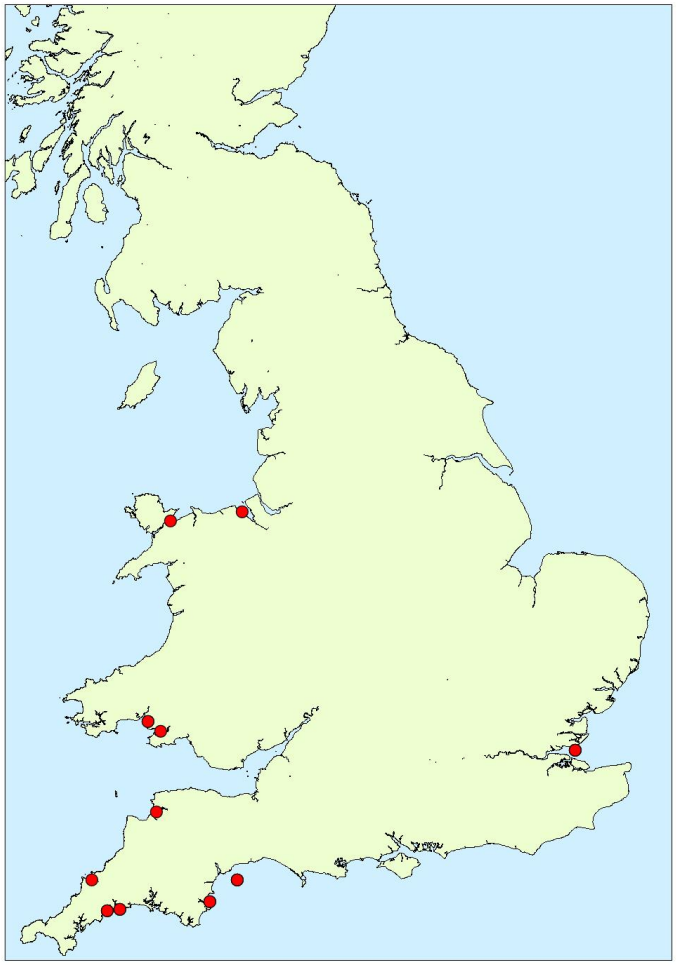


Figure 13: Locations of sites where *Dinophysiaceae* were detected above trigger level in 2017

Figure 14: Locations of sites where *Prorocentrum lima* was detected above trigger level in 2017

2. Biotoxin sample results in 2017

2.1. Amnesic Shellfish Poisoning (ASP) and *Pseudo-nitzschia* species

No samples recorded ASP toxins above the trigger level of 10µg/g.

Table 4. Summary of *Pseudo-nitzschia* species detected above the trigger level of 150,000 cells/L. Results ordered by Local Authority.

Production Area	Bed ID	Local Authority	Sampling Point	Date Sample Collected	*Pseudo-nitzschia* spp. cells L-1
Burry Inlet	B038D	Carmarthenshire CC	Machynys	01/06/2017	181000
Burry Inlet	B038D	Carmarthenshire CC	Machynys	13/06/2017	373000
Camel	B35AE	Cornwall CC	Porthilly Rock B	16/05/2017	222000
Fowey	B70AB	Cornwall PHA	Pont Pill	04/07/2017	330000
Fowey	B70AB	Cornwall PHA	Pont Pill	10/07/2017	838000
St Austell Bay	B70AE	Cornwall PHA	Ropehaven Outer	04/07/2017	280000
St Austell Bay	B70AE	Cornwall PHA	Ropehaven Outer	10/07/2017	437000
St Austell Bay	B70AE	Cornwall PHA	Ropehaven Outer	15/08/2017	757000
Lantivet Bay	B70AH	Cornwall PHA	Sandheap Point	04/07/2017	264000
Lantivet Bay	B70AH	Cornwall PHA	Sandheap Point	10/07/2017	226000
Lantivet Bay	B70AH	Cornwall PHA	Sandheap Point	15/08/2017	893000
Dart	B028B	South Hams DC	Waddeton	24/04/2017	169480

Table colour coding: Green (above causative algae trigger level)

2.2. Paralytic Shellfish Poisoning (PSP) and *Alexandrium* species

Table 5. Summary of PSP toxins and *Alexandrium* spp. detected above the trigger levels of 400 µg STXeq/kg and 40 cells/L respectively during 2017. Results ordered by Local Authority.

Production Area	Bed ID	Local Authority	Sampling Point	Date Sample Collected	High value calculated from method uncertainty	*Alexandrium* spp. cells L-1
Morecambe Bay - Barrow	B077Q	Barrow-in-Furness BC	Roa Island	05/07/2017		40
Three Rivers	N/A	Carmarthenshire CC	Kidwelly Quay	25/07/2017		100
Burry Inlet	B038D	Carmarthenshire CC	Machynys	01/06/2017		40
Burry Inlet	B038D	Carmarthenshire CC	Machynys	06/06/2017		100
Burry Inlet	B038D	Carmarthenshire CC	Machynys	20/06/2017		40
Burry Inlet	B038D	Carmarthenshire CC	Machynys	02/08/2017		40
Fal	B33AN	Cornwall PHA	Mylor Creek	31/07/2017		80

Fal	B33AN	Cornwall PHA	Mylor Creek	08/08/2017		40
Percuil	B033R	Cornwall PHA	Percuil	27/03/2017		200
Fowey	B70AB	Cornwall PHA	Pont Pill	06/06/2017		80
Fowey	B70AB	Cornwall PHA	Pont Pill	19/07/2017		80
Fowey	B70AB	Cornwall PHA	Pont Pill	01/08/2017		40
Fowey	B70AB	Cornwall PHA	Pont Pill	01/08/2017	1590	
Fal	B033Y	Cornwall PHA	Ruan Pontoon/Tregothnan	25/07/2017		160
Fal	B033Y	Cornwall PHA	Ruan Pontoon/Tregothnan	08/08/2017		80
Fal	B33BD	Cornwall PHA	Trelissick Pontoon	25/07/2017		80
Fal	B33BD	Cornwall PHA	Trelissick Pontoon	06/09/2017		40
Chichester Harbour	B018Q	Havant BC	Northney Marina	10/07/2017		40
Crouch	B015Y	Maldon DC	Althorne	26/07/2017		100
Blackwater	B014V	Maldon DC	Goldhanger	09/10/2017		80
Beaulieu	B023I	New Forest DC	Landing Stage	31/05/2017		100
Beaulieu	B023I	New Forest DC	Landing Stage	14/06/2017		40
Yealm	B031F	Plymouth PHA	Thorn	21/06/2017		120
Yealm	B031J	Plymouth PHA	Thorn	14/08/2017		80
Yealm	B031J	Plymouth PHA	Thorn	24/08/2017		40
Yealm	B031J	Plymouth PHA	Thorn	20/09/2017		100
Yealm	B031J	Plymouth PHA	Thorn	09/08/2017		120
Poole	B54CL	Poole BC	West Brownsea 1	05/06/2017		40
Salcombe	B029D	South Hams DC	Geese Quarries	11/04/2017		100
Salcombe	B029D	South Hams DC	Geese Quarries	25/04/2017		80
Salcombe	B029D	South Hams DC	Geese Quarries	09/05/2017		100
Salcombe	B029D	South Hams DC	Geese Quarries	24/05/2017		100
Salcombe	B029D	South Hams DC	Geese Quarries	09/08/2017		760
Salcombe	B029D	South Hams DC	Geese Quarries	09/08/2017	831	
Salcombe	B029D	South Hams DC	Geese Quarries	22/08/2017	1713	
Salcombe	B029D	South Hams DC	Geese Quarries	06/09/2017		2100
Salcombe	B029D	South Hams DC	Geese Quarries	06/09/2017	434	
Salcombe	B029D	South Hams DC	Geese Quarries	20/09/2017		200
Dart	B028B	South Hams DC	Waddeton	07/06/2017		40
Solent	B24BK	Southampton PHA	Browndown	17/07/2017		40
Southampton Water	B021Y	Southampton PHA	Hamble Estuary	05/06/2017		80
Southampton Water	B021Y	Southampton PHA	Hamble Estuary	12/06/2017		40
Southampton Water	B021Y	Southampton PHA	Hamble Estuary	14/08/2017		100

Swansea	B037U	Swansea PHA	Queens Dock	03/05/2017	40
Swansea	B037U	Swansea PHA	Queens Dock	09/05/2017	240
Exe	B26BH	Teignbridge DC	Cockwood Harbour	16/08/2017	40
Brixham	B082B	Torbay BC	Fishcombe Cove SW Corner	10/04/2017	40
Brixham	B082B	Torbay BC	Fishcombe Cove SW Corner	03/05/2017	40
Taw/Torridge	B36AB	Torridge DC	Spratt Ridge East	01/06/2017	80
Taw/Torridge	B36AB	Torridge DC	Spratt Ridge East	13/06/2017	40
Taw/Torridge	B36AB	Torridge DC	Spratt Ridge East	18/07/2017	40
Porlock	B094D	West Somerset Council	Porlock East	23/05/2017	100
The Fleet	B25AI	Weymouth PHA	Fleet Oysters	17/05/2017	40
The Fleet	B25AI	Weymouth PHA	Fleet Oysters	20/06/2017	40

Table colour coding: Yellow (above toxin trigger level), Red (above toxin MPL), Green (above causative algae trigger level)

2.3. Lipophilic Toxins, *Dinophysiaceae* and *Prorocentrum lima*

Table 6. Summary of results for samples recording Lipophilic toxins; Okadaic acid (OA), Dinophysistoxins (DTXs) and Pectenotoxins (PTXs), above the trigger level of 80 µg OA eq/kg. The table also includes samples recording the LTs causative algal species (Dinophysiaceae and *Prorocentrum lima*), above the trigger level of 100 cells/L. Results ordered by Local Authority. (Please note; toxin and algal results are only shown when the trigger levels have been breached).

Production Area	Bed ID	Local Authority	Sampling Point	Date Sample Collected	Total OA/DTXs/PTXs (µg OA eq/kg) High value calculated from MU	*Dinophysieae* cells L-1	*Prorocentrum lima* cells L-1
Three Rivers	N/A	Carmarthenshire CC	Kidwelly Quay	19/07/2017		100	
Three Rivers	N/A	Carmarthenshire CC	Kidwelly Quay	25/07/2017		100	
Burry Inlet	B038D	Carmarthenshire CC	Machynys	13/06/2017		100	
Conwy	B044V	Conwy CBC	Conwy West	26/07/2017			200
Camel	B35AE	Cornwall CC	Porthilly Rock B	31/01/2017		200	
Fowey	B70AB	Cornwall PHA	Pont Pill	05/12/2017		120	
St Austell Bay	B70AE	Cornwall PHA	Ropehaven Outer	17/05/2017		120	
St Austell Bay	B70AE	Cornwall PHA	Ropehaven Outer	06/06/2017		160	
Lantivet Bay	B70AH	Cornwall PHA	Sandheap Point	17/05/2017		120	
Lantivet Bay	B70AH	Cornwall PHA	Sandheap Point	14/06/2017		320	

Dee	B45AB	Flintshire CC	Salisbury	09/08/2017		100	
Menai Strait East	B055S	Gwynedd CC	West of Bangor Pier	31/05/2017		120	
The Thames	B16BR	London PHA	Phoenix	07/08/2017		160	
The Thames	B16BR	London PHA	Phoenix	21/08/2017		120	
The Thames	B16BR	London PHA	Phoenix	05/09/2017		160	
Holy Island-Ross Links	B001M	Northumberland CC	Ross Links	10/07/2017			100
Brixham	B082B	Torbay BC	Fishcombe Cove SW Corner	28/06/2017		120	
Brixham	B082B	Torbay BC	Fishcombe Cove SW Corner	05/07/2017		120	
Lyme Bay	B090M	Torbay BC	Site 1	27/06/2017		160	
Lyme Bay	B090M	Torbay BC	Site 1	04/07/2017		160	
Lyme Bay	B090M	Torbay BC	Site 1	12/07/2017		200	
Lyme Bay	B090M	Torbay BC	Site 1	12/07/2017	236		
Lyme Bay	B090M	Torbay BC	Site 1	20/07/2017	213		
Lyme Bay	B090M	Torbay BC	Site 1	25/07/2017	234		
Lyme Bay	B090M	Torbay BC	Site 1	01/08/2017	162		
Taw/Torridge	B36AB	Torridge DC	Spratt Ridge East	12/07/2017		120	

Table colour coding: Red (above toxin MPL), Green (above primary causative algal species trigger level), Grey (above secondary causative algal species trigger level)

No samples contained Azaspiracids (AZAs) above the trigger level of 80 µg AZA1 eq/kg during 2017.

No samples contained Yessotoxins (YTXs) above the trigger level of 1.875 mg YTX eq/kg during 2017.

3. Results of the 2017 wild pectenidae verification programme

Up until 31st of March 2017, samples of wild pectenidae were collected by 2 local authorities from auction houses, processing plants and/or dispatch centres. As the samples are not collected from designated monitoring points, information on the origin of the samples was taken from the shellfish movement document by the LA collecting the sample. Their approximate origins are indicated in Figure 15.

Three whole scallop samples were submitted for ASP analysis and the results are summarised in Table 7 below.

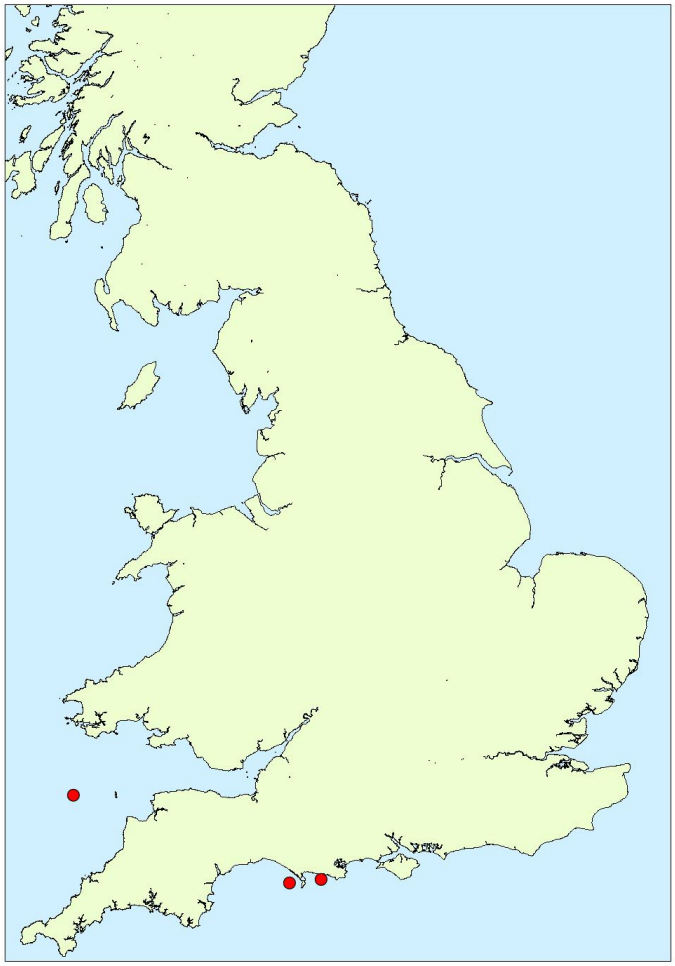


Figure 15: Approximate origins of wild pectenidae samples collected in 2017

Table 7: Results of the 2017 wild pectenidae verification programme (England & Wales)

Local Authority	Sample composition	No of samples submitted	No of unsuitable samples	ASP detected (>MPL)
Weymouth PHA	Whole King scallops	2	0	0
Pembrokeshire	Whole King scallops	1	0	1

4. Glossary

AOAC	AOAC International
ASP	Amnesic Shellfish Poisoning
AZA	Azaspiracid
AZP	Azaspiracid Poisoning
Cefas	The Centre for Environment, Fisheries and Aquaculture Sciences
DA	Domoic Acid
DSP	Diarrhetic Shellfish Poisoning
DTX	Dinophysistoxin
dcSTX	decarbomoyl Saxitoxin
EC	European Commission
EU	European Union
EURL	European Union Reference Laboratory for Marine Biotoxins
EHO	Environmental Health Officer
FSA	Food Standards Agency
GTX	Gonyautoxin
HPLC	High Performance Liquid Chromatography
LA(s)	Local Food Authority(ies)
LC-MS	Liquid Chromatography – Mass Spectrometry
LTs	Lipophilic toxins
MPL	Maximum permitted limit
N/A (na)	Not Applicable
ND	Not Detected
OC	Official Controls
OA	Okadaic Acid
PSP	Paralytic Shellfish Poisoning
PST	Paralytic Shellfish Toxins
PTX	Pectenotoxin
PTX2sa	Pectenotoxin 2 seco acid
7-*epi* PTX2sa	7-epi-Pectenotoxin 2 seco acid
RL (<RL)	Reporting Limit
SOP(s)	Standard Operating Procedure(s)
STX	Saxitoxin
UKNRL	UK National Reference Laboratory for Marine Biotoxins
YTX	Yessotoxin

5. 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) 854/2004 of the European Parliament and of the Council of 29 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.

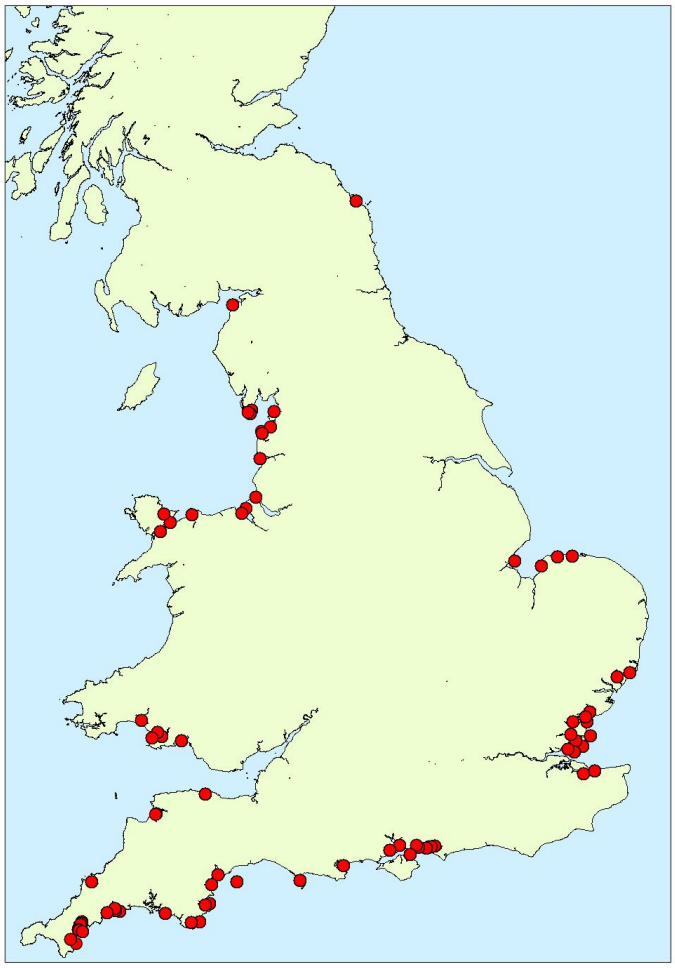
European Communities (2004). Regulation (EC) 853/2004 of the European Parliament and of the Council of 29th April 2004 laying down the specific hygiene rules for the hygiene of foodstuffs.

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

Appendix 1 – Methodology for official control monitoring of toxins in shellfish

A. *Shellfish collection and transport*

In 2017, 39 local authorities (LAs) contributed to the sampling of shellfish from 76 inshore locations (Appended figure 1).



Appended figure 1: English and Welsh <u>flesh</u> sampling locations - Biotoxin monitoring programme 1st January to 31st December 2017

In total, 862 shellfish samples were submitted from classified production and relaying areas. Samples were submitted for amnesic shellfish poisoning (ASP) toxins, paralytic shellfish poisoning (PSP) toxins and/or lipophilic toxins (LTs) testing. Environmental Health Officers (EHOs) from Local Authorities (LAs) collected or supervised the collection of shellfish samples from designated monitoring points within classified shellfish production or relaying areas. The samples received from classified production and relaying areas comprised mainly of mussels (*Mytilus* spp.), native oysters (*Ostrea edulis)*, common cockles (*Cerastoderma edule)* and Pacific oysters (*Crassostrea gigas)* (Appended table 1). The remainder of the samples consisted of surf clams (*Spisula solida*), razor clams (*Ensis* spp*)*, manila clams (*Tapes philipinarum)* and hard clams (*Mercenaria mercenaria*).

Shellfish samples reached Cefas between 1 and 72 hours post collection, with 91% of samples reaching the lab within 1 working day and 100% reaching the lab within 2 working days.

Shellfish samples were transported to the testing laboratory using a validated chilled transport system (Coleman 16 Qrt coolboxes). Over 95% of the samples transported in these boxes arrived at the laboratory within the recommended temperature range (2-10°C). Thirty-nine samples recorded temperatures between 10.1 and 18.9°C. However, upon inspection, all of these samples met the criteria set by the UK Marine Biotoxins National Reference Laboratory (UKNRL) for testing.

B. Shellfish sample assessment

Unsuitable samples

On arrival at the laboratory, samples were assigned a unique laboratory number and their temperature recorded before they were assessed for their suitability for analysis, in accordance with UKNRL SOPs. Shellfish which failed to respond to a percussion test and/or did not exhibit organoleptic characteristics associated with freshness were excluded from testing and reported as unsuitable for analysis.

Samples were assessed on the basis of their compliance with the requirement of the monitoring programme (namely; shellfish species submitted, frequency of submission and geographical origin of the sample). Samples taken from non-active sites or unclassified species were queried with the LA. If no suitable reason was provided, then the sample was rejected. Three samples were rejected on the grounds of being submitted outside the scheduled testing frequency.

Insufficient samples

Samples which were assessed as suitable for analysis were then prepared for ASP, PSP and/or lipophilic toxins analyses as required. In accordance with agreed procedures, should the amount of shellfish available provide insufficient material for all required tests, prioritisation of analyses is based on the historic prevalence of toxin group or lack of previous monitoring results for any toxin group at each site. Where no information is available or prioritisation cannot be ascertained on the above criteria, PSP toxin analyses are prioritised over LT and ASP analyses. No samples were found to be insufficient for the required tests in 2017.

Appended table 1. Summary of samples received and found insufficient/unsuitable for ASP, PSP or lipophilic toxins analyses, by species, in 2017.

Species	Total no. of samples submitted for analysis	No. of samples found insufficient for any of the required tests	No. of samples found unsuitable	No. of samples found unsuitable due to location or frequency	Percentage of samples found insufficient/unsuitable for the required tests (%)
Mussels	405	0	0	1	0.2
Pacific oysters	221	0	0	1	0.5
Native oysters	52	0	0	0	0
Common cockles	95	0	0	1	1.1
Surf clams	12	0	0	0	0
Manila clams	24	0	0	0	0
Hard clams	53	0	0	0	0
TOTAL	*862*	0	0	3	0.3

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.
- 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 characteristics and 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.

Appended table 2: 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

Test outcome

Samples were considered as positive if they were found to breach the maximum permitted limits (MPL) for marine toxins specified in EC regulation 853/2004 (Table 1).

Where these levels were exceeded, recommendations were for temporary harvesting restrictions to be put in place for all shellfish species classified in the affected area until two consecutive negative or below action level (action level equals MPL) results were achieved for the toxin which was the cause of the closure, and at least one further negative or below action level result for the toxin groups which had not exceeded the MPL.

Routine flesh testing frequencies were defined by the FSA and followed one of three set plans:

1. **Areas with a historic risk of PSP toxins occurrence AND/OR have insufficient historic data**.
   Fortnightly from 1st of April to 30th of September
   Four weekly from 1st of October to 31st of March

2. **Areas with no historic risk of PSP toxins AND historic data**
   Four weekly throughout the year

In addition, requests were made for weekly shellfish monitoring to be instigated when set trigger levels, indicative of heightened toxicity risk were breached. The trigger levels used in the 2017 reporting period are summarised in Appended table 4:

Appended table 4: Flesh trigger levels

Toxin group	Levels of toxin or cell concentrations triggering additional monitoring if breached
ASP	≥10mg domoic/epi-domoic acid/kg shellfish flesh
LTs	OA/DTX/PTX group: ≥80 µg OAeq./kg shellfish flesh AZA group: ≥80 µg AZA1eq./kg shellfish flesh YTX group: ≥1.8mg/kg shellfish flesh
PSP	≥400µg STX eq./kg shellfish flesh

D. Reporting of results

Upon completion of the required analyses, the results were collated and quality controlled prior to submission to FSA. Results were reported on a daily basis. A summary of results turnaround times, from day of receipt to completion of each analysis for 2017 is given in Appended table 5. For reference, the turnaround times agreed with the FSA and required from Cefas during the reporting period are given in Appended table 6.

Appended table 5: Turnaround times, by test carried out, for samples received from classified production and relay areas in 2017

Territory	No. of tests performed	No. of completed results reported within one working day of receipt of sample	No. of completed results reported two working days post receipt of sample	No. of completed results reported three working days post receipt of sample
ASP by HPLC	746	745 (>99%)	1 (<1%)	0
Lipophilic toxins by LC-MS	762	748 (98%)	13 (1.7%)	1 (<1%)
PSP by HPLC (screen)	833	832 (>99%)	1 (<1%)	0
PSP by HPLC (quantitation)	4	0	4 (100%)	0
Totals	**2345**	**2325 (>99%)**	**19 (<1%)**	**1 (<1%)**

Appended table 6: Sample turnaround times (from sample receipt) specified by FSA

Toxin and analysis method	FSA specified targets
ASP by HPLC	90% within 1 working day 98% within 3 working days
Lipophilic toxins by LC-MS	90% within 1 working day 98% within 3 working days
PSP by HPLC (screen/semi-quantitation)	90% within 1 working day 100% within 3 working days
PSP by HPLC (quantitation)	80% within 2 working days 100% within 4 working days

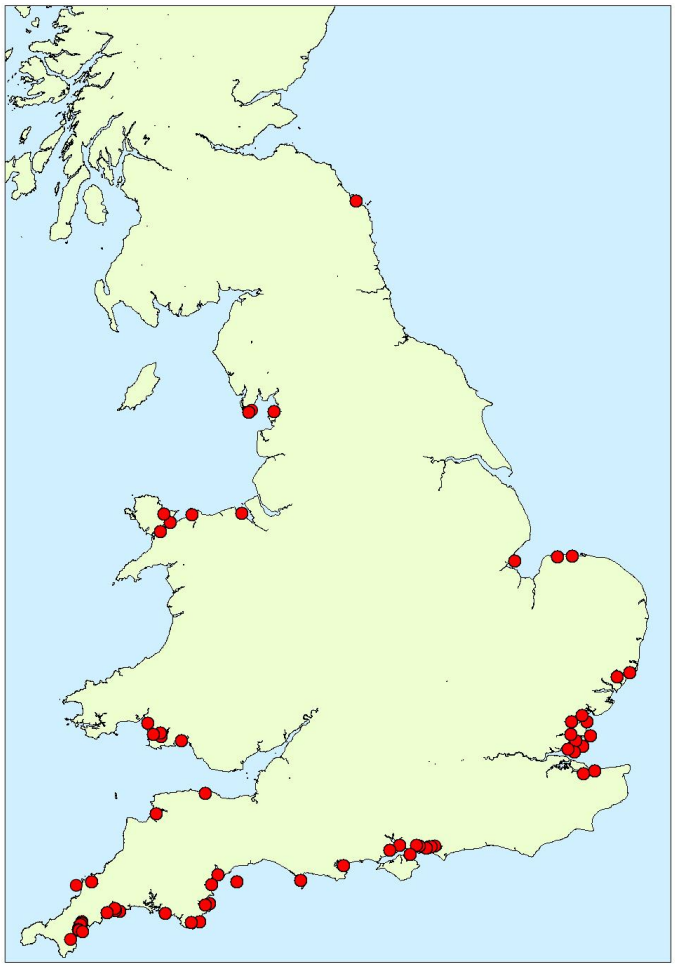
Required turnaround times were therefore all met and for all analyses, delivery by the laboratory exceeded the targets agreed with FSA.

In addition to the daily reporting schedule, all results from samples received between Monday and Friday the previous week were collated and reported in a weekly results sheet to FSA, released the following week.

Appendix 2 – Methodology for official control monitoring of toxic phytoplankton in classified shellfish production areas

A*. Phytoplankton sample collection and transport*

921 phytoplankton samples were collected by environmental health officers from 52 classified production or relaying areas around the coast of England and Wales (Appended figure 1).



Appended figure 1. English and Welsh <u>water</u> sampling locations – 2017
Biotoxin monitoring programme

Sample collectors were requested to take depth integrated water samples from above the harvesting areas, at high water when possible. Tube samplers were provided to local authority staff who had access to boats, or where piers and jetties were sufficiently close to the flesh sampling points to allow a depth integrated sample to be taken. However, it was recognised that their use was not always practical in shallow, coastal areas and a homogenised sample, collected from three depths (near bottom, midwater and near surface) using a pole sampler, was recommended as a preferential alternative to sampling surface water with a bucket.

A 500mL nalgene bottle was filled with water from each sample collection, which was preserved with the addition of 2mL of acidified Lugol's Iodine. Preserved samples were then posted in pre-paid special delivery bags, together with a completed sample collection form, to the Cefas plankton laboratory for analysis.

B. Assessment of sample suitability

On arrival at the laboratory, samples were assigned a unique laboratory number. Sub-samples were then set up in 25mL Utermöhl chambers and allowed to settle. After three hours each sample was given a preliminary examination. If the viewing area contained too much sediment, then an additional sub-sample was set up in a 10mL or 5mL Utermöhl chamber. All samples were allowed to settle for a minimum of 12 hours before the final suitability assessment was made. If after 12 hours, the viewing area of the smaller chamber was also obscured by sediment then these samples were reported as "unable to analyse" in the weekly results sheet.

A total of 9 samples (0.94%) were rejected, most of these (n=6, 0.6%) were due to high sediment concentrations in the water. This is a further decrease on the previous year's figures, of which 19 (1.7%) were rejected due to high sediment concentrations. This reflects ongoing efforts by Cefas and the collectors to resolve issues in some locations by either changing the sampling location or the sampling method. Three other samples were rejected which were submitted outside of the routine testing frequency.

C. Water sample analysis

Water analyses followed the standard operating procedures drawn up by the UK national reference laboratory for marine biotoxins. Phytoplankton analyses are accredited to ISO 17025:2005 standard.

<u>Test outcome</u>

'Trigger levels' remained at the same cell concentrations as in previous years (Appended table 1). When these levels were breached, the FSA was immediately contacted and requests were made for additional water and shellfish samples to be collected. These were submitted for analysis the following week. When shellfish flesh samples breached trigger levels the water sampling was suspended (for the first time this year) until such time as toxin levels in the flesh fell below the trigger level.

Appended table 1: Trigger levels for toxin producing algae

Toxin	Toxin producing algae (trigger Level)
ASP	*Pseudo-nitzschia spp.* (150,000 cells/L)
LTs	*Dinophysiaceae* (100 cells/L) *Prorocentrum lima* (100 cells/L)
PSP	*Alexandrium spp.* (Presence)

D. Reporting of results

Upon completion of analyses, the results were collated and quality control checked prior to submission to the FSA. During 2017, Cefas was able to report all results within one working day of sample receipt. This turnaround time is in full compliance with the targets specified by the FSA which is set at 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 which was released to the FSA by the following week.