Centre for Environment Fisheries & Aquaculture Science

Cefas

Testing of crustacean tissue samples associated with NE England mortality events for regulated marine biotoxins – May 2022 samples

Cefas analysis following reports of crustacean sickness - North East England, May 2022

REDACTED

June 2022

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This report provides a brief summary of the results obtained following the testing of crab and lobster tissue samples received at Cefas on 26th May 2022 from NE IFCA. This follows on from previous reports which showed low/trace concentrations of some marine toxins in crabs from the NE coast together with a fairly consistent presence of diarrhetic shellfish toxins. For this study:

- Lobsters were received from 15 different sampling points or fishing vessels, crabs from 7. Sample collection/receipt and sample shipment was conducted by NE IFCA.
- Between 1-6 animals were provided for each "sample" – so each sample represents the pooled contents of between 1-6 animals, from the same sampling point.
- Where animals provided were large enough, after shucking, the meat was separated into "brown" and "white" tissue and analysed separately (the exception being 3 "whole" crab samples, as the animals sampled were too small).
- **Table 1** summarises the information received with the samples.
- Testing was conducted for the regulated marine neurotoxins - ASP toxins (domoic acid) and PSP toxins (saxitoxins), as well as the diarrhetic lipophilic toxins, comprising Diarrhetic shellfish poisoning toxins (DSP – okadaic acid, dinophysistoxins and their esterified counterparts), azaspiracids (AZAs), pectenotoxins (PTXs) and yessotoxins (YTXs).
- The method used for detection of PSP toxins has been fully validated for application to crabs, but no such validation has been performed for ASP, DSP and other lipophilic toxins. As such, these results are not reported to ISO17025 standard. Methods used for sample testing are summarised in **Table 2**.

Table 1. Summary of samples received showing sample information provided by IFCA

Cefas id	Tissue	Ref number	Species	Location/vessel	Date landed	n
BLC 1	White	PO3921935	lobster	Redcar	29-Apr	1
	Brown					
BLC 2	White	PO3921827	lobster	Fleet-6	09-May	1
	Brown					
BLC 3	White	PO3922004	lobster	Redcar	24-May	2
	Brown					
BLC 4	White	PO3921823	lobster	Redcar	09-May	3
	Brown					
BLC 5	White	PO3921832	lobster	South Gare	29-Apr	3
	Brown					
BLC 6	White	PO3921817	lobster	Sea mist hand dived (54, 407 6 N, 001, 11, 0066 W)	09-May	1
	Brown					
BLC 7	White	PO3921833	crab	Huntcliff	05-May	3
	Brown					
BLC 8	White	PO3921833	lobster	Huntcliff	05-May	1
	Brown					
BLC 9	White	PO3922003	lobster	Redcar	24-May	2
	Brown					
BLC 10	White	PO3921832	crabs	Good intent (54:36:30N, 032:00W)	no date on bag	4
	Brown					
BLC 11	Whole	PO3921830	crabs	Saltburn	02-May	5

BLC 12	White	PO3921828	lobster	Fleet-2 (Hullpool)	09-May	1
	Brown					
BLC 13	White	PO3921835	lobster	South Gare	29-Apr	3
	Brown					
BLC 14	Whole	PO3921835	crab	South Gare	29-Apr	1
BLC 15	White	PO3921882	lobster	Dominator	28-Apr	1
	Brown					
BLC 16	White	PO3921937	lobster	Redcar	29-Apr	1
	Brown					
BLC 17	White	PO30922138	lobster	Ad Noble	20-May	1
	Brown					
BLC 18	White	PO30922138	crab	Ad Noble	20-May	1
	Brown					
BLC 19	White	PO3921829	lobster	Hartlepool	28-Apr	1
	Brown					
BLC 20	Whole	PO3921821	crab	Dungeon hole	16-May	6
BLC 21	White	PO3921822	crab	Dungeon Hole	16-May	3
	Brown					
BLC 22	White	PO3921836	lobster	Redcar	29-Apr	2
	Brown					

Table 2. Summary of methods utilised for testing:

Analytes	Method	Status
Paralytic shellfish toxins (PSTs)	Acetic acid extraction, carbon de-salting SPE clean-up, dilution and LC-MS/MS analysis	Validated in shellfish and crab, but not accredited
Domoic acid (DA)	50% aqueous methanol (MeOH) extraction with LC-UV analysis	Developmental method, performance characteristics determined in crab, not accredited
Lipophilic toxins (LT) including Diarrhetic Shellfish Toxins (DST)	100% methanolic extraction followed by LC-MS/MS analysis	Validated in shellfish but not for non-bivalves

Results

- PSP toxins – no toxins detected in any sample.
- ASP – domoic acid detected at low concentrations (0.4 to 4.2 mg/kg) in ~50% samples. The majority of positive samples were white meat. We suspect these are false positives, resulting from unknown matrix components affecting the detection. Further work would be required using mass spectrometry to confirm this (previously mass spec showed this to be the case with samples from Sept-Oct). Even so, levels quantified are less than 25% of the maximum permitted limits for ASP in bivalve molluscs, and crabs are known in other parts of the world to accumulate levels of ASP many hundreds of times higher than this, without any obvious adverse health effects.

- Lipophilic toxins by LC-MS/MS – only trace levels of DSP toxins (okadaic acid and dinophysistoxin 2) detected in a low number of samples, all at levels below our normal limit of quantitation. Azaspiracids (AZAs) detected and quantified at higher levels in ~50% of samples. In some samples the total AZA content approached half the MPL for AZA. Yessotoxin was detected at trace levels in only two samples.
- **Table 3** summarises the results obtained following each of the methods undertaken at the Cefas laboratory. The full results for each sample are listed in the associated Excel table "Crustacean toxin results summary – for sharing – NE crustacean May 2022 samples.xlsx".

Table 3. Overall summary of results for each class of toxins

Analytes	**Findings**	**Comments**
Paralytic shellfish toxins (saxitoxins) by LC-MS/MS	No PSP toxin analogues detected in any samples	LODs range from 0.4 to 13 µg STX eq/kg per analogue for shellfish
Domoic acid by LC-UV	~41% of samples containing peaks indicative of domoic acid with maximum toxin concentration reaching 4.2 mg/kg (<25% of MPL = 20 mg/kg)	LOD estimated around 0.4 mg/kg
Diarrhetic shellfish poisoning (DSP) toxins by LC-MS/MS	~20% of samples containing trace levels of DSP toxins (okadaic acid and dinophysistoxin 2 ; OA/DTX2 <10 µg/kg (<10% of MPL = 160 µg/kg)	LOD estimated around 1-5 µg/kg
Other regulated lipophilic toxins by LC-MS/MS	~50% samples containing AZA1 above LOD, with some also containing AZA2. Maximum concentration 50 µg/kg AZA1 eq/kg (32% of the MPL of 160 µg/kg). ~5% of samples containing trace concentrations of yessotoxin (YTX), maximum concentration of 10 µg/kg (~0.25% of MPL)	LOD estimated around 1-5 µg/kg

[a]STX, dcSTX, NEO, dcNEO, C1-4, GTX1-6, dcGTX1-4

Note that following dissection of the whole lobsters provided, eight samples showed the clear presence of the parasitic lobster louse *Nicothose astaci* in the gills, which is linked to respiratory issues within lobsters.

To note:

- These methods are targeted detection methods, i.e. they can only detect specific compounds which are incorporated into the method(s) and are available as certified reference standards. We are not able to conduct non-targeted screening assays – other organisations should be consulted if this approach is required.
- As discussed previously, these methods are validated only in the matrix of bivalve mollusc shellfish (various species), and crab (for PSP toxins only). As such, we have no evidence for toxin recovery and method performance for the analysis of domoic acid in these samples. Consequently, there is the potential for under or over-estimating toxin concentrations, without any such validation of method performance characteristics.

Conclusions

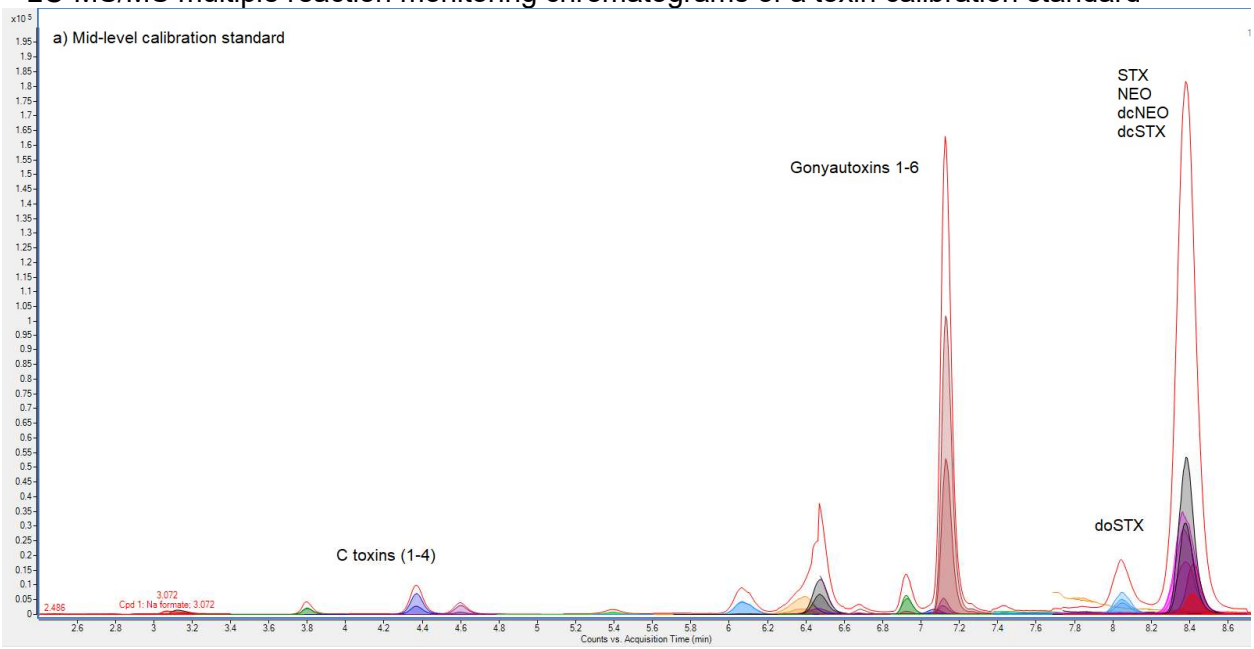
- Overall, there was an absence of any high concentrations of regulated marine toxins present in any samples, with AZAs being present at the highest concentrations, noting currently there is no information as to whether AZAs impact upon crustacean health.
- It is stressed that we do not have any “baseline data” for marine toxin levels in crustacean from healthy animals around the UK coastline, at different times of the year.
- We would recommend this baseline screening to be conducted in crustacean from around the coast to enable future robust statistical assessment of unexpectedly high toxin levels, especially if ongoing animal health issues are expected.

Annex – methods used for analysis

1. *PSTs (saxitoxins) by LC-MS/MS*

Extraction of tissues using 1% acetic acid (in boiling water) as per validated method Turner et al., 2020. Centrifuged extracts subjected to desalting step and dilution in acetonitrile, prior to analysis by LC-MS/MS.

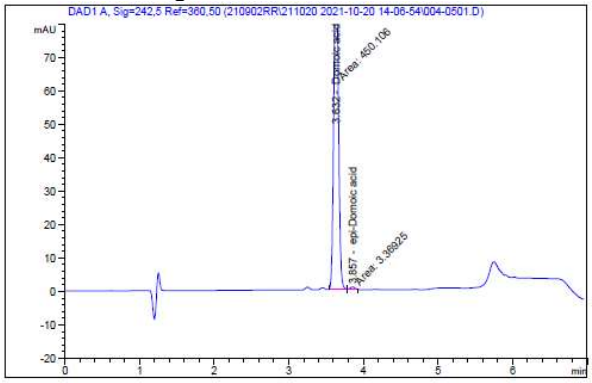
LC-MS/MS multiple reaction monitoring chromatograms of a toxin calibration standard



2. *DA by LC-UV*

Samples were extracted using 50% MeOH / 50% Water, using the approach described above. After extraction, centrifuged supernatants were filtered (0.2 µm) and subjected to LC with UV detection (LC-UV) without SPE as conducted for routine monitoring of bivalves.

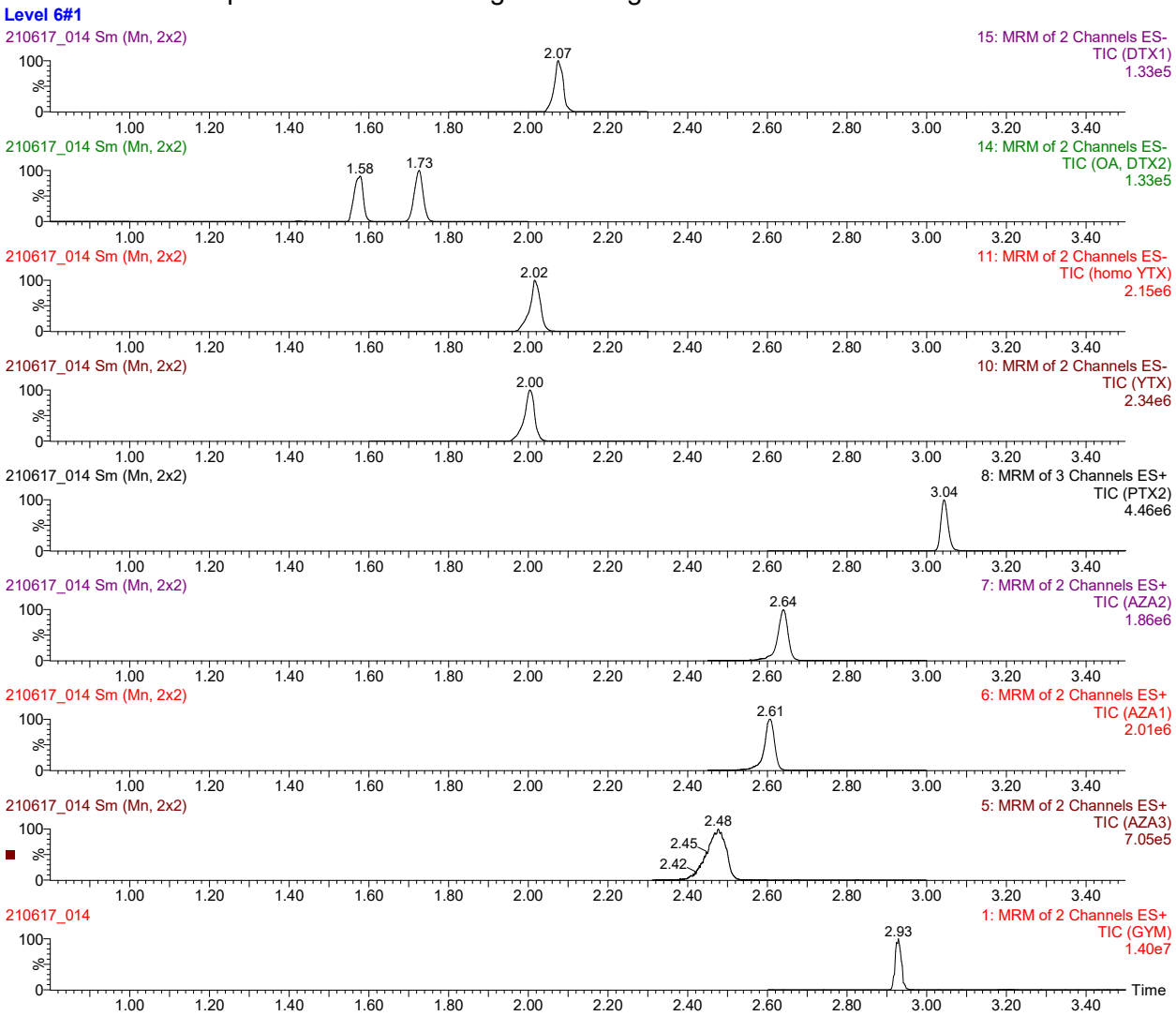
UV chromatogram of domoic acid standard



3. *Lipophilic toxins by LC-MS/MS*

LC-MS/MS following EU reference laboratory reference method. Extraction of tissues using 100% methanol prior to filtration and LC-MS/MS analysis. An additional aliquot subjected to alkaline hydrolysis to liberate acyl esters of OA-group toxins prior to additional LC-MS/MS analysis.

LC-MS/MS multiple reaction monitoring chromatograms of toxin standards



References

[1] Turner, A.D., Dhanji-Rapkova, M., Fong, S.Y.T., Hungerford, J., McNabb, P.S., Boundy, M.J. and Harwood, D.T. (2020). Ultrahigh-performance hydrophilic interaction liquid chromatography with tandem mass spectrometry method for the determination of paralytic shellfish toxins and tetrodotoxin in mussels, oysters, clams, cockles and scallops: collaborative study. *J. AOAC International.* 103, 1-35

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Cefas

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We work in partnership with our colleagues in Defra and across UK government, and with international governments, business, maritime and fishing industry, non-governmental organisations, research institutes, universities, civil society and schools to collate and share knowledge. Together we can understand and value our seas to secure a sustainable blue future for us all and help create a greater place for living.

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